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CONTROLLING INFLUENCES IN CORN ROT PROBLEMS

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(WITH THREE FIGURES)

Introduction

A statement issued by the Office of Mycology and Disease Survey of the Bureau of Plant Industry (11) indicates that the more important corn-producing states estimated a loss of about 10 per cent of the corn yield for 1926 as a result of root, stalk, and ear rots. HOLBERT and his co-workers (5) suggest a greater loss for the state of Illinois. Crop reports have indicated that the annual loss from corn rots amounts to several million dollars each year.

Plant pathologists have not generally agreed as to a definite limitation of corn rot diseases. The tendency has been to associate some fungus with each pathogenic condition. Ear rots are supposed to be caused by *Diplodia zeae* (Schw.) Lev., stalk rots by *Fusarium moniliforme* Sheldon, root rots and seedling blights by *Gibberella saubinetii* (Mont.) Sacc., scutellum rots by *Rhizopus* spp., and black bundles by *Cephalosporium acremonium;* also some bacteria have been suspected of causing rots. The fungi have been found by various investigators in all parts of the corn plant.

All seem to agree, however, that corn suffers from certain diseased conditions associated with symptoms of a definite nature. Rotting of the roots, weakening and discolorations of the nodes, seed-

ling blight, red streaking of leaves or paleness, weakness of the ear shank, barren stalks, and chaffy kernels, have all been designated as indications of greatest importance. In the field, a very irregular stand and "pale spots," varying in size from a few square rods to several acres in extent, are common. These spots are readily recognized in the older fields of the Corn Belt, east of the Mississippi River. The stalks may range from 2 to 4 feet in height, with a pale color and a tendency to lodge; while the corn about these places will be much taller and of a rich green color. An examination of the individual stalks in these spots will reveal the symptoms commonly known as root rot.

The fact that these spots are surrounded by apparently vigorous corn, often producing high yields, causes one to wonder whether the fungi are the cause of such well defined spots, or whether there is not some ecological or physiological factor controlling the extent of the disease. If it were limited entirely to pathogenic organisms, it appears that the disease would be distributed over the entire field. The problem here undertaken has been to find causes for these pale spots, and, if possible, to suggest a means of eliminating them.

Historical data

In 1889 BURRILL (2), describing a bacterial disease of corn, mentioned several symptoms commonly attributed to corn rots. He found the roots badly decayed in many cases, the stalks discolored at the lower nodes, streaking of the leaf sheath, and a decay of ears. A "close, very white, feltlike fungus" was mentioned as subsequently infecting the ears. BURRILL states that the disease was first called to his attention in 1882.

Due to poisoning of farm animals by a "cornstalk disease" in Nebraska, interest was revived in the study of corn infection by "molds." In 1903, SHELDON (10) described *Fusarium moniliforme* as an organism producing a pink mold of corn. A similar motive prompted a study of *Diplodia zeae* by VAN DER BIJL (13) in South Africa, in 1916.

During the past few years, more intensive studies have been made by investigators in the agricultural experiment stations of the Corn Belt. One group believes that whatever rot is present is due

to a parasitic fungus; a second group holds the more progressive viewpoint that susceptibility is based upon genetic weaknesses of the corn plant; and a third group supports the idea that malnutrition and a lack of physiological balance are important factors in causing the corn rots.

HOFFER and his co-workers (3) have perhaps advanced the last view more vigorously than any others. They claim that excessive accumulations of iron in the nodes are accompanied by a lack of potassium obtained from the soil, and that a deficiency of nitrogen in the stalk follows a decrease of potassium in the soil. These weakened stalks are said to be increasingly susceptible to disease (4). Microchemical tests applied at the nodes of cornstalks have been recommended as soil indicators for deficiencies of potash and nitrogen. WELTON and others (14) showed that such indicators are unreliable in Ohio. They found the tests decidedly variable, and advised the farmers of that state against the use of such tests for selecting fertilizers, because they are "frequently conflicting and often misleading and unreliable."

HOLBERT and his associates (5) have prepared an exhaustive report of their investigations of the resistance of various strains of corn to fungous infection. They show that it is possible to select a type of corn which is decidedly resistant to infection. The hard, smooth type, with "proper indentation" is shown to be more desirable than the rough starchy type. Their conclusions suggest that earlier plantings are more susceptible to scutellum rot than later ones, and that seedling blights are more common in cooler soils. Selection and crop rotations are indicated as their final means of controlling disease.

VALLEAU (12) has recently shown that a soil-borne fungus, described as a *Pythium*-like organism, is the source of corn root rots. *Gibberella*, *Fusarium*, and *Diplodia* species are secondary, perhaps causing a seedling blight, according to his account. Where continuous cropping in corn is practiced, the infection is said to be greater.

Experimental methods

Several experiments in greenhouses and in the field were conducted to determine the influences of soils and chemicals upon diseased and disease-free corn. These were begun at the University of

Chicago in 1922. Later they were extended to fields in Indiana and in Oklahoma. The five experiments outlined summarize the work done from 1922 to 1927 inclusive.

Influences of iron and aluminium

Control cultures of corn were grown to determine the influences of iron and aluminium upon the composition of the corn plant, in the presence and in the absence of a fungus. For this experiment, *Diplodia zeae* was used as a fungus causing typical corn rots. It was most readily isolated from the diseased corn obtained.

An attempt was made to secure corn that was known to be free from pathogenic infection. The varieties Dakota Yellow Dent, Madison Yellow Dent, Longfellow (flint), Reid's Yellow Dent (northern grown), and a strain of 90-Day Corn were obtained from a Chicago seed house. Germination tests showed very slight infection. Samples of seeds from each variety were soaked in distilled water for 12 hours, covered for 3 minutes with a 10 per cent solution of silver nitrate, washed with a N/10 solution of NaOH, and finally washed with sterile distilled water. These grains were then dropped into deep germinating dishes containing sterile dextrose agar. Due to the severe sterilization, only about 10 per cent of the kernels germinated. Since fungi and bacteria did not develop upon the seedlings nor upon the nutrient agar, it may be assumed that the corn used in subsequent studies was reasonably free from pathogenes at the time of transplanting.

Sand pots were used for cultural studies. Eight-inch earthenware pots were coated with paraffin and filled with clean white sand which had been treated for 24 hours with sulphuric acid. The pots of sand were then washed with running water for 12 hours and sterilized in a steam bath for 4 hours.

Stock solutions of nutrient salts were made up separately. Molecular solutions of magnesium sulphate, calcium nitrate, and potassium dihydrogen phosphate were made in sufficient quantities for the entire experiment. Baker "analyzed" chemicals and distilled water were used. Solution type I, R_3S_2, diluted three and one-half times as recommended by the Committee of the National Research

Council on Salt Requirements of Representative Agricultural Plants, was made for keeping the sand moist and for the nutrition of the corn. The amounts of the salt solution varied from time to time, but an effort was made to apply the same amount to all pots.

When the hypocotyls had reached a length of 0.5 inch, the seedlings were transplanted from the germinating dishes to the sand pots. Each variety of corn was represented by 5 pots: one pot of each variety was set aside as a control series; a second series was inoculated at the cotyledonary node; a third series was treated with a 0.0005 M. solution of aluminium sulphate; a fourth series with a 0.0005 M. solution of aluminium chloride; and a fifth series with a 0.0005 M. solution of ferrous sulphate. The iron and aluminium salts were added to their respective series three times each week for 6 weeks.

After the corn had matured the plants were removed from the pots, the sand washed from the roots, and the total green weights obtained. The plants were then air-dried. A quantitative estimation of the amounts of aluminium and iron was made of the roots, nodes, and internodes of each plant, according to the Official Methods (8). The 3 lower nodes of each stalk were used in each case.

Influence of fungi upon yield

One corn plot was used for 3 years in comparing the yields of diseased and good seed. Beginning in 1922, diseased corn was planted upon 5 acres and good corn was planted upon the adjoining 5 acres, and the experiment was repeated upon the same field during 1923 and 1924. The grain was drilled in rows 3.5 feet apart at the rate of one grain to each foot. The field was a very rich river bottom in southern Indiana. Two hundred and fifty pounds of 2–12–6 fertilizer was added annually to each acre. Perhaps the fertility and soil conditions were as good as might be obtained for corn production.

Seeds for the experiment were obtained by testing with a "modified-rag-doll." Ears that showed no signs of fungous infection during germination were considered good seed; those which showed infection by various fungi, but which were completely viable, were kept for planting the diseased plot. The same procedure was followed

each year. Yields were estimated by weighing each load of corn upon a farm scale as it was husked, in November. Conditions of tillage were the same.

Influences of temperature and fertilizers

During the 1925 season, an experiment was conducted to determine the influences of temperature and climate upon corn rots. Rich sand-loam was available in northern Indiana and in southern Oklahoma. From soil surveys and from general appearances, these soils were almost identical in composition, the differences being in temperature and moisture. New soil thermographs, regulated identically

TABLE I

Planting plan of field plots

Row applications	Indiana corn	Oklahoma corn	Show corn
1. Potash	50 gm. per hill	50 gm. per hill	50 gm. per hill
2. Chilean nitrate	25 gm. per hill	25 gm. per hill	25 gm. per hill
3. Cottonseed meal	1 lb. per hill	1 lb. per hill	1 lb. per hill
4. Ferrous ammonium sulphate	10 gm. per hill	10 gm. per hill	10 gm. per hill
5. Control	.....	.....	.....
6, 7. Gibberella saubinetii	Sprayed suspension	Sprayed suspension	.....
8, 9. Diplodia zeae	Sprayed suspension	Sprayed suspension	.....
10. Control	.....	.....	.....

by Julien P. Friez of Baltimore, were used to record the temperatures in the fields. The Oklahoma field had grown three crops of cotton but had never been planted to corn since the original prairie sod was broken. The Indiana corn followed a heavy clover crop, where the corn-wheat-clover rotation had been practiced for 50 or 60 years. Both fields were plowed at the same depth and received the same amount of cultivation. Twenty-five rows of corn were planted in each plot, by the ear-to-row method. The first 5 ears in each plot were from a sample of Indiana corn which had placed third in the International Grain and Hay Show, at Chicago. They were of the Reid Yellow Dent variety, and represented the best type of show corn available. A germination test revealed no sign of fungous infection in any of these 10 ears, and the germination was perfect. Twenty ears of Reid's Yellow Dent, free from signs of fungous infection, were selected from seed that had been naturalized to the Red River Valley of Oklahoma. Another 20 ears of corn were selected

from that grown in northern Indiana. Thus each plot contained 10 rows of Indiana corn, 10 of Oklahoma corn, and 5 rows of "show" corn. Each row was 40 rods in length. Estimations of yields were made upon a basis of 20 rows making an acre. Estimations of yield were made in Oklahoma on July 24; those in Indiana on October 5. Table I indicates the applications of chemicals and fungi which were made upon these duplicate plots. They were made when the two lots of corn were at the same stages of development. The Oklahoma season was more than one month in advance of the Indiana season.

Influences of moisture and soil texture

An experiment was conducted to determine the influences of moisture and soil texture upon the composition of the corn plant and upon its susceptibility to disease. Forty 5-gallon tin oilcans were used; 20 were filled with a heavy yellow clay, and 20 with rich garden soil. Ten of each were planted with a disease-resistant strain of corn, and the remaining cans were planted with susceptible corn. The seed was kindly provided by Dr. G. N. Hoffer of Purdue University, and both strains had been inbred for several generations. The grains were surface-sterilized and germinated upon agar plates to make sure that no fungi were present when the experiment started. Ten of the clay cans and 10 of the loam cans were watered daily to maintain an excess of moisture; the others were watered only enough to keep the corn growing.

After the corn had been planted three weeks, 5 gm. of ferrous ammonium sulphate, dissolved in one liter of distilled water, was added to one can in each of the four groups. The treatment was repeated weekly for 5 weeks. Aluminium sulphate was added in the same amounts to four other pots at the same time. Applications of fungous cultures were made to cans in each group by spraying the corn with water suspensions. When the first coleoptiles began to appear above the ground spraying was begun. A second application was made when the stalks were 6 inches high.

The corn was harvested at the time of tasseling, and weights and analyses were recorded. Quantitative estimations of reducing sugars, total nitrogen, ferric and aluminium oxides, and phosphorus were made, according to the Official Methods (**8**).

Influences of colloidal soil

An experiment was begun in the greenhouses of the University of Chicago to determine the influences of a colloidal silt loam upon the development of roots, and upon the susceptibility of corn to infection from fungi. The work was suggested by observations made during several trips across the Corn Belt. Practically all the "pale spots" in corn fields are found in places where the clay is inclined to

TABLE II

Planting plan for corn grown in cans

Can no.	Wet clay	Dry clay	Wet loam	Dry loam
1	Ferrous ammonium sulphate	Ferrous ammonium sulphate	Ferrous ammonium sulphate	Ferrous ammonium sulphate
2	Aluminium sulphate	Aluminium sulphate	Aluminium sulphate	Aluminium sulphate
3	Diplodia zeae	Diplodia zeae	Diplodia zeae	Diplodia zeae
4	Gibberella saubinetii	Gibberella saubinetii	Gibberella saubinetii	Gibberella saubinetii
5	Control	Control	Control	Control

TABLE III

Arrangement of soils and treatment

Soil treatment	Funk's disease-free seed		Infected seed	
	Silt loam (gm.)	Greenhouse soil (gm.)	Silt loam (gm.)	Greenhouse soil (gm.)
Ferrous ammonium sulphate	10	10	10	10
Lime	25	25	25	25
Manure	200	200	200	200
Sodium nitrate	10	10	10	10
Potassium chloride	10	10	10	10
Control				

"puddle," or where the drainage is poor, producing what the farmers call "cold places." Clermont silt loam was obtained from a field which indicated these conditions. The lime requirement for general agricultural purposes was determined by the Soiltex indicator. Lamotte and potentiometer estimations were made of the pH concentration. The percentage of colloidal material in the soil was estimated by the use of the most recent hydrometer method of Bouyoucos (1).

Twelve glazed pots containing silt loam, and 12 containing the best greenhouse soil were steam-sterilized for 8 hours. Surface-sterilized grains of Funk Brothers' "inbred, high-oil, disease-free, utility type" of corn were germinated upon sterile agar in Petri dishes and planted in 6 pots of each kind of soil. A second planting was made with corn from the same source which was known or was thought

TABLE IV A

Treatment	Total green weight	Total dry weight	Total ash per cent dry weight	Al_2O_3 total dry weight		Fe_2O_3 total dry weight	
				Nodes	Roots	Nodes	Roots
	Longfellow (flint)						
Control	387	132	2.1	0.029	0.014	0.009	0.015
Infected	278	63	3.4	0.019	0.011	0.008	0.0094
	90-Day						
Control	252	113	2.02	0.007	0.0074	0.019	0.018
Infected	19	6	3.21	0.004	0.0052	0.010	0.018
	Reid's Yellow Dent (northern)						
Control	252	113	2.21	0.0037	0.0058	0.008	0.017
Infected	107	27	2.71	0.0031	0.0056	0.005	0.013
	Madison Yellow Dent						
Control	387	132	2.1	0.0036	0.0148	0.004	0.017
Infected	178	53	3.4	0.0025	0.0112	0.003	0.014
	Dakota Yellow Dent						
Control	222	94	2.07	0.0038	0.0132	0.004	0.013
Infected	96	27	3.5	0.002	0.0067	0.003	0.009

to be infected with *Gibberella saubinetii*. The grains were surface-sterilized and germinated upon sterile agar. Those grains which were viable but which showed signs of *Gibberella* infection were planted in the remaining clay and greenhouse soil pots. Treatments of the respective groups are shown in table III.

Five kg. of soil was used in each pot. With the exception of the manure, which was applied dry, the chemicals were applied by dissolving the amounts indicated in one liter of water and applying at the rate of 100 cc. daily until the entire amounts had been added.

A soil temperature of 21° was maintained, and water was added daily. The chemist of the Ohio Agricultural Experiment Station, Wooster, Ohio, estimated that the silt loam contained the following nutrients: nitrogen 0.154 per cent, phosphorus 0.40 per cent, potassium 2.07 per cent.

The root systems were washed out carefully, and the dry weights of the entire plants were recorded at the end of the experiment.

TABLE IV B

Total green weight	Total dry weight	Total per cent ash	$Al_2(SO_4)_3$				$Fe_2(SO_4)_3$				Al_2Cl_6			
			Al_2O_3		Fe_2O_3		Al_2O_3		Fe_2O_3		Al_2O_3		Fe_2O_3	
			Node	Root	Node	Root	Node	Root	Node	Root	Node	Root	Node	Root
Longfellow														
405	73	2.1	0.218	4.9	0.129	0.34	.....	.....	.....	.....	.....	.....	.....	.....
371	53	2.38	.....	.....	.....	.....	0.236	0.23	0.149	0.48	.....	.....	.....	.....
486	69	2.54	.....	.....	.....	.....	.....	.....	.....	.....	0.291	1.78	0.084	0.24
90-Day														
377	145	1.7	0.56	4.04	0.114	0.228	.....	.....	.....	.....	.....	.....	.....	.....
287	94	2.5	.....	.....	.....	.....	0.190	0.243	0.23	0.83	.....	.....	.....	.....
436	109	3.1	.....	.....	.....	.....	.....	.....	.....	.....	0.19	0.62	0.140	0.237
Reid's Yellow Dent (northern)														
282	101	2.5	0.402	1.17	0.271	0.19	.....	.....	.....	.....	.....	.....	.....	.....
293	74	2.1	.....	.....	.....	.....	0.24	0.34	0.291	0.211	.....	.....	.....	.....
332	121	3.7	.....	.....	.....	.....	.....	.....	.....	.....	0.38	1.14	0.22	0.204
Madison Yellow Dent														
356	81	1.8	0.329	0.31	0.26	0.24	.....	.....	.....	.....	.....	.....	.....	.....
406	140	2.3	.....	.....	.....	.....	0.29	0.169	0.306	0.258	.....	.....	.....	.....
391	128	2.5	.....	.....	.....	.....	.....	.....	.....	.....	0.34	0.205	0.29	0.205
Dakota Yellow Dent														
137	47	1.99	0.236	0.74	0.246	0.21	.....	.....	.....	.....	.....	.....	.....	.....
231	68	2.4	.....	.....	.....	.....	0.216	0.182	0.214	0.331	.....	.....	.....	.....
232	73	3.08	.....	.....	.....	.....	.....	.....	.....	.....	0.34	1.93	0.207	0.182

Experimental data

Influences of iron and aluminium

The corn planted in the sand pots grew rapidly. Stalks from each variety, inoculated with *Diplodia zeae*, began to show signs of infection within a week. The characteristic red and brown streaks were

evident. No marked difference was noted in the susceptibility of the five varieties. Pure cultures of the fungus were obtained from the inoculated stalks at various intervals during the experiment.

Stalks treated with aluminium chloride, aluminium sulphate, and ferrous sulphate did not show symptoms of the root and stalk rots, but the growth was rather more vigorous than the controls. Quantitative estimations of the first three nodes and of the roots were made of all stalks for the amounts of ferric and aluminium oxides present. These are summarized in tables III and IV. The tabulations also show the green and dry weights, as well as the ash content.

INFLUENCE OF FUNGI UPON YIELDS

Table V summarizes the yields of disease-free and of diseased corn upon river bottom land during a period of three years. As previously stated conditions were very favorable for growing corn during the entire period.

TABLE V

SUMMARY OF YIELDS FOR 3 YEARS

YEAR	FIVE ACRES GOOD SEED (BUSHELS)	FIVE ACRES DISEASED SEED (BUSHELS)
1922	401	405
1923	389	406
1924	360	452
3-year total	1150	1263

Yields were estimated by weight of corn taken from the field. A bushel was considered 72 lb.

INFLUENCES OF TEMPERATURE AND FERTILIZERS

The soil temperatures of Oklahoma and Indiana cannot be compared from the point of view of corn growth. During the summer of 1925 Indiana had almost an ideal corn season, while Oklahoma did not. No rain fell in the latter state after June 15 until the end of the experiment. The scorching sun and hot winds reduced the yield almost to nothing. It is interesting to note that early varieties of corn, planted in March, matured sufficiently early to avoid the hot weather and produced a fair crop, while the later varieties almost perished.

The yields given in table VIII were estimated by weighing the ears produced upon 40 rods. Twenty such rows are considered an acre; 72 lb. is considered a bushel.

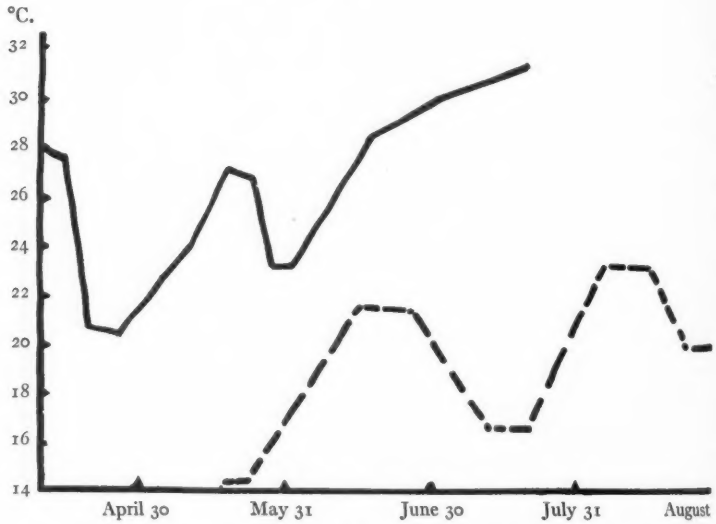


FIG. 1.—Graphic comparison of Oklahoma and Indiana soil temperatures: vertical column represents range of temperatures (°C.); horizontal column indicates time; solid line indicates Oklahoma temperatures; dotted line represents Indiana temperatures.

TABLE VI

YIELDS OF INDIANA REID'S YELLOW DENT IN OKLAHOMA AND IN INDIANA

ROW NO.	TREATMENT	INDIANA YIELD PER ACRE (BUSHELS)	OKLAHOMA YIELD PER ACRE (BUSHELS)
1	Potash	71	5
2	Chilean nitrate	69	3.5
3	Cottonseed meal	67	4
4	$FeSO_4(NH_4)_2SO_4 \cdot H_2O$	70	3
5	Control	73	6
6, 7	Gibberella saubinetii	71 and 69	7 and 8
8, 9	Diplodia zeae	64 and 68	5 and 3.5
10	Control	72	4
Average yield		69.4	4.9

Influences of moisture and soil texture

The following data were obtained by weighing the entire corn plants after washing and drying to a constant weight. These plants were grown in the 5-gallon cans of soil.

TABLE VII

Yields of Oklahoma Reid's Yellow Dent in Oklahoma and in Indiana

Row no.	Treatment	Indiana yield per acre (bushels)	Oklahoma yield per acre (bushels)
1	Potash	43	6
2	Chilean nitrate	47	7
3	Cottonseed meal	40	3
4	$FeSO_4 \cdot (NH_4)_2SO_4 \cdot H_2O$	38	2
5	Control	45	9
6, 7	Gibberella saubinetii	46 and 42	4 and 5
8, 9	Diplodia zeae	39 and 44	7 and 3
10	Control	46	6
Average yield		43	5.2

TABLE VIII

Yields of show corn in Indiana and Oklahoma

Row no.	Treatment	Indiana yield per acre (bushels)	Oklahoma yield per acre (bushels)
1	Potash	62	12
2	Chilean nitrate	63	2
3	Cottonseed meal	58	7
4	$FeSO_4(NH_4)_2SO_4 \cdot H_2O$	64	9
5	Control	61	11
Average yield		61.6	8.2

TABLE IX

Comparative weights of corn plants grown from resistant seed

Can no.	Treatment	Clay		Garden soil	
		Wet	Dry	Wet	Dry
		Gm. dry weight	Gm. dry weight	Gm. dry weight	Gm. dry weight
1	$FeSO_4(NH_4)_2SO_4 \cdot H_2O$	246	102	420	163
2	$Al_2(SO_4)_3$	257	75	450	139
3	Diplodia zeae	191	69	301	168
4	Gibberella saubinetii	97	104	275	155
5	Control	262	109	450	313

In the preceding tables the terms wet and dry refer to conditions of the soil; dryness means about 40 per cent of the water-holding capacity of the soil; wet, 60 per cent.

Analyses were made of the plants indicated, estimating quantitatively the constituents listed in table XI. For iron and aluminium

TABLE X

COMPARATIVE WEIGHTS OF CORN PLANTS GROWN FROM SUSCEPTIBLE SEED

CAN NO.	TREATMENT	CLAY		GARDEN SOIL	
		Wet	Dry	Wet	Dry
		Gm. dry weight	Gm. dry weight	Gm. dry weight	Gm. dry weight
1	$FeSO_4(NH_4)_2SO_4 \cdot H_2O$	229	107	390	104
2	$Al_2(SO_4)_3$	241	91	405	113
3	Diplodia zeae	173	62	349	88
4	Gibberella saubinetii	96	56	192	52
5	Control	194	86	381	120

TABLE XI

CHEMICAL COMPOSITION OF RESISTANT CORN

CONSTITUENTS	CLAY		GARDEN SOIL	
	Wet	Dry	Wet	Dry
	Per cent dry weight	Per cent dry weight	Per cent dry weight	Per cent dry weight
Reducing sugar	1.76	1.95	1.85	1.31
Phosphorus	0.123	0.096	0.23	0.14
Nitrogen	0.79	0.62	0.89	0.48
Ferric oxide	0.05	0.047	0.07	0.058
Aluminium oxide	0.135	0.21	0.097	0.106

determinations the three nodes just above the ground were taken. These were dried to a constant weight, ashed, and the estimations made from the ash. The remainder of the plant was ground as finely as possible with a food chopper and preserved in alcohol, made up to 80 per cent.

INFLUENCES OF COLLOIDAL SOIL

Clermont silt loam and the greenhouse soil were tested to determine the limestone requirement for general agricultural purposes and for the pH values. Table XIII also indicates the percentage of

colloids present in the respective soils, as determined by the Bouyoucos hydrometer method (1).

The corn grown in the 24 pots of this experiment (table XIII) was harvested early, in order to make determinations for a more

TABLE XII

CHEMICAL COMPOSITION OF SUSCEPTIBLE CORN

CONSTITUENTS	CLAY		GARDEN SOIL	
	Wet	Dry	Wet	Dry
	Per cent dry weight	Per cent dry weight	Per cent dry weight	Per cent dry weight
Reducing sugar	2.11	2.22	1.86	2.08
Phosphorus	0.151	0.091	0.113	0.083
Nitrogen	0.625	0.341	1.102	0.89
Ferric oxide	0.029	0.080	0.075	0.064
Aluminium oxide	0.181	0.310	0.121	0.154

TABLE XIII

ACIDITY AND COLLOIDAL PERCENTAGE IN SOILS

METHOD USED	SILT LOAM	GREENHOUSE SOIL
Soiltex	3 tons limestone	Neutral
LaMotte comparator	pH value 5.8	pH value 6.6
Potentiometer	pH value 4.5	pH value 6.71
Hydrometer colloid content	25.3 per cent	17.5 per cent

TABLE XIV

YIELDS ON SILT LOAM AND GREENHOUSE SOILS EXPRESSED IN GM. OF DRY WEIGHT OF CORN

SOIL TREATMENT	FUNK'S DISEASE-FREE SEED		INFECTED SEED	
	Silt loam	Greenhouse	Silt loam	Greenhouse
Ferrous ammonium sulphate	22.4	54.7	33.8	45.2
Lime	11.5	35.6	18.5	41.3
Manure	34.5	59.1	37.0	57.8
Sodium nitrate	17.9	51.8	25.6	53.3
Potassium chloride	41.7	58.6	34.0	53.4
Control	20.4	43.9	18.5	53.0

extensive field experiment. The corn had grown for 6 weeks, and had reached a height of 2 feet when it was removed from the pots and washed. It was air-dried and weighed as shown in table XIV.

Discussion of results

In the sand cultures, treatment of the growing corn with solutions of aluminium chloride, aluminium, and ferric sulphates did not produce a pathogenic condition similar to the root, stalk, or ear rots of corn. The sulphates of iron and aluminium seemed to improve the growth. A number of quantitative estimations were made of the nodes and roots of diseased and control stalks of corn, to determine the comparative amounts of aluminium and iron compounds present. The former usually contained more of these elements than did the controls.

The 3-year test of yields of diseased and disease-free seed was suggested by an extensive seed corn testing program conducted by the writer during the seasons of 1921 and 1922, in a southern Indiana county. Skeptical farmers questioned the elimination of corn diseases by selection with rag-doll germinators, then in vogue in Indiana. Suggestions were offered that a comprehensive experiment be conducted under the direction of the writer. Tests were made of the seed and the planting was supervised as outlined. The yield is not unusual, considering the fact that some of the best seed and best methods of cultivation were used. MELCHERS and JOHNSTON (7) confirm the results of this experiment. They show that a large percentage of the seed ordinarily used is infected with *Fusarium moniliforme*, and that seedling vigor is more important than the absence of fungi.

Observing the two plots of corn, it was impossible to detect any difference in the vigor of the stalks. The fact that the diseased corn produced a few more bushels during the 3 years was probably due to the fact that the stand was not so heavy. There undoubtedly was some seedling blight, which eliminated a few of the weaker stalks, giving an opportunity for the development of larger ears. The stand of the disease-free corn was thicker, and the ears did not grow so large, because of overcrowding.

It may be concluded that as long as fungous infection does not affect seed viability to a great extent, the yield is not decreased.

While the Oklahoma corn yields were almost a failure, some interesting facts were shown in connection with the limits of temperatures at which corn will produce a crop. Until the tasseling stage,

the Oklahoma field was more promising of definite results than that in Indiana. The applications of Chilean nitrate caused the second rows of each planting to grow almost a foot taller than the others. All other applications had no visible effect upon the growth of stalks. On June 15, an extremely hot, dry wind began to blow, at the time when the ears were forming. Within a few days the entire field had turned to the sickening brown characteristic of Oklahoma landscapes at that time of year. The corn which had been treated with Chilean nitrate developed a very pronounced case of root and stalk rot during the process. The nodes developed discolorations, the stalks broke of their own weight, and the roots decayed. Frequent attempts to isolate *Diplodia* and *Gibberella* from these stalks failed. A bacterium which seems to cause a vegetable rot was present in nearly every case. It might have been similar to that described by ROSEN (9) in Arkansas.

Judging from yields, frequent sprayings of plants with suspensions of spores of the fungi did not produce disease. Large quantities of the fungi had been grown upon cornmeal, in large Erlenmeyer flasks, for the purpose of spraying. Field applications of fungi generally associated with corn rots did not produce symptoms attributed to the diseases, if the plants were growing vigorously. HOLBERT and others (5) have emphasized this fact in their reports.

The influences of soil texture and moisture content were investigated under more controlled conditions. Yellow clay soil did not produce the growth naturally expected from garden soil, and the stalks were apparently much more susceptible to infection from *Diplodia* and *Gibberella*. Stalks were sprayed rather frequently and the usual symptoms developed. Infection was less pronounced in the garden soil. Root rots were evident to a greater extent in the dry cans that in the wet. It is interesting to note that the "resistant" corn was diseased as much as the "susceptible" in this experiment. The fact that both had been inbred in opposite directions was not an appreciable factor. The former had a more nearly normal kernel, and naturally produced a more vigorous stalk, as shown by the controls.

Iron and aluminium sulphates did not have an injurious effect upon corn. In the wet clay the growth was more vigorous than in

the controls. The leaves were a darker green. It is probable that these salts had a tendency to flocculate the clay. In making the analysis of the lower nodes of the stalks, it was found that corn growing in heavy clay soils has a higher aluminium and iron content than corn growing in sandy loams. This does not seem to be associated with susceptibility to diseases directly, but is related rather to assimilation. The phosphorus content was greater in corn grown in garden soil, and the nitrogen content was also higher. HUME (6) states that the ash constituents of corn plants do not correlate with a normal nor an abnormal condition, but differ with individual plants.

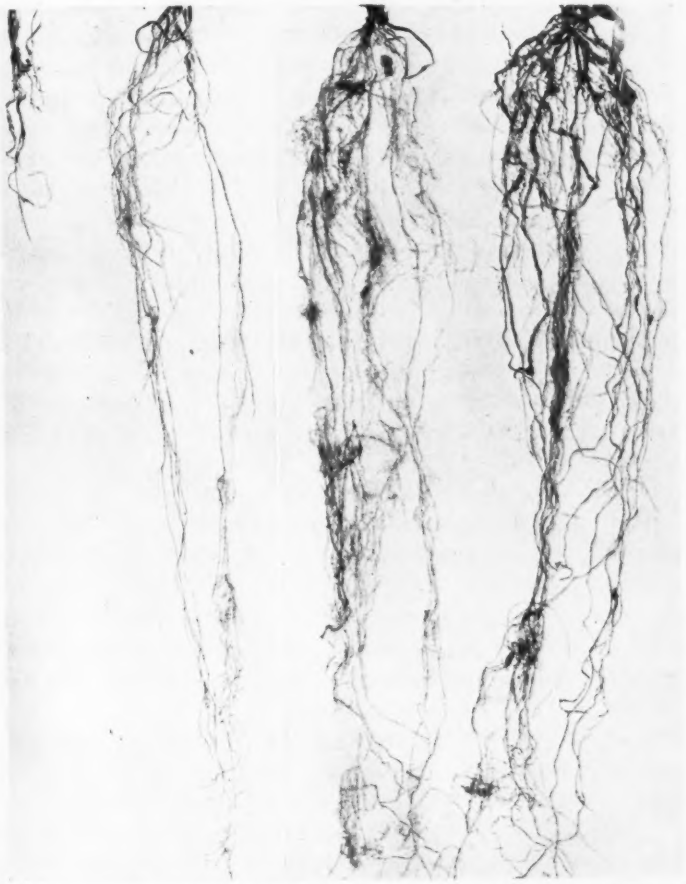
From this experiment, it is evident that the physical and chemical conditions of the soil are of greater importance in the development of corn plants than the presence or absence of fungi.

Following the suggestions of soil influences which were noted in this experiment in Oklahoma, the writer had an opportunity to observe the spotted condition of diseased cornfields during several trips through the leading corn-producing states. The "puddled soils" or "gumbo" produced diseased corn, and the "pale spots" are usually limited to these areas.

A silt loam, commonly known as white clay, was obtained to demonstrate the lack of proper aeration in the soil. It showed a limestone requirement of 3 tons per acre. With a LaMotte comparator set, the pH value was estimated at 5.8. A potentiometer reading of a suspension of the same soil indicated a pH value of 4.5. The hydrometer methods of colloid determination indicated that the silt loam had a colloidal content, almost 50 per cent greater than that of greenhouse soil.

Applications of materials were made to the pots of soil, to determine which would provide the best flocculant for field experiments. The aim was to use those correctives which are within the range of agricultural practice. From the dry weights of the plants it is quite evident that potassium chloride and manure are the most successful in producing vegetative growth. While lime corrected the acidity, it did not improve the growth but rather injured the corn. Sodium nitrate might well be expected to prove beneficial, but it did not.

Ferrous ammonium sulphate had been found to be a very effective flocculant of the clay, and it was used for that purpose. Along with



FIGS. 2, 3.—Fig. 2 (left), finely branched roots of a plant grown in colloidal soil; coarser roots grown in greenhouse soil; fig. 3 (right), roots of corn plants inoculated with *Gibberella saubinetii;* badly decayed roots grew in colloidal soil, others grew in greenhouse soil.

potassium, it may have had some nutritive value which would account for the increased production. Comparisons were made by a

duplicate experiment with Funk's best seed corn and with seed from the same source, which was known to be infected with *Gibberella saubinetii*. Under the same conditions the growths were similar.

Washing the soil away from the roots revealed a marked difference in the branching of the root systems. Those grown in silt loam were much branched and finely divided. Since the experiment was conducted with sterilized soil, it is quite probable that the much branched root is more susceptible to rot infections than those coarser roots from the loose garden soil. A detailed study of the methods of infection in corn seedlings shows that lesions occur at points where the secondary roots break through the cortex. With an abnormal development of secondary branches, it is evident that such corn plants will show root rots more readily than those which have the normal branching. Fig. 2 shows this difference in branching. Fig. 3 shows the difference in response to inoculation with a pure culture of *Gibberella saubinetii*. The larger root growing in greenhouse soil shows some infection, but the smaller plant shows almost complete decay.

Summary

1. Under optimum conditions for corn production, if the seed is viable, fungous infection of the type usually called root rot does not affect the yield.

2. Extremely high soil temperatures do not increase the susceptibility of corn to diseases, although the corn may be killed by the extreme heat. The Oklahoma soil temperature approached the thermal death point of maturing corn.

3. Applications of iron and aluminium salts do not increase the susceptibility of corn to diseases. In the case of clay soils, a limited application seems to be beneficial, perhaps as a flocculant.

4. Colloidal soils induce an extraordinary development of secondary roots in corn plants and render them more susceptible to fungous infection. Lesions originate at points where secondary roots break through the cortex.

5. A large percentage of corn rots may be eliminated by flocculating colloids in the soil, allowing the normal development of the plant.

The writer desires to acknowledge his appreciation of the valuable and timely suggestions of Dr. C. A. SHULL and Dr. S. V. EATON of the Department of Botany of the University of Chicago. Those farmers who have contributed so generously of their land and labor deserve much credit for the results of the experiments.

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SEED GERMINATION IN CERTAIN NEW MEXICO RANGE GRASSES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 385

CAROLA V. JACKSON

(WITH FIVE FIGURES)

Introduction

Much work has been carried on in recent years in regard to the viability and germination of seeds of various plants. Seed-testing studies are highly important, because the results obtained influence or affect the work of the farmer, the floriculturist, the amateur gardener, and the rancher or ranger. These people cannot afford to plant seeds, expecting a 95 per cent germination and then perhaps having only a 50 per cent germination, due to poor selection of seed, to its immaturity, or to its adulteration with weed seeds.

Among the plants studied for germination the grasses have a prominent part, and this is especially true of the range grasses of the west. It is necessary for the rancher or ranger to know about what percentage of the seeds of the grasses covering his grazing lands he can expect to germinate, since he can then estimate the amount of grazing his lands will tolerate without becoming depleted. He must know whether his grasses propagate themselves vegetatively or by seed, or by both means.

Various studies have been made with reference to seed germination, and although they have no direct bearing upon the problem which the writer undertook, yet they gave ideas for some tests which were performed after the major problem was completed. The following are short summaries of the work of some of the investigators in the field of seed germination.

In general it is believed that the delay in after-ripening is due to the characters of the embryo and of the seed coat. Miss ECKERSON (6) found that there is a series of metabolic changes going on in the embryo during the period of after-ripening. At first the acidity is increased, and correlated with it is increased activity of catalase

and peroxidase. By treating the embryos with dilute acids such as hydrochloric, butyric, and acetic, the after-ripening period can be reduced very much. Those embryos which are treated increase their water-holding power, acidity, and amount of peroxidase more rapidly; and the oxidase appears sooner than in the untreated embryo.

PAMMEL and KING (14) planted mature and immature seeds in the fall and in the spring. In general, stratification in sand and freezing were favorable to germination. *Asclepias syriaca* showed 12 per cent germination, *Ambrosia psilostachya* 18 per cent, *Chenopodium album* 88 per cent, and *Xanthium canadense* 25 per cent.

PACK (13) discovered that the germination of non-after-ripened *Juniperus* seeds under ordinary conditions is very small, amounting to 1 per cent. These seeds are protected by a semi-permeable and thick coat which makes up 75 per cent by weight of the entire seed. Acids enter very slowly, he found, while bases, silver, and mercury salts enter rapidly. PACK thinks that while the coat may act as a protection against fungal attack, and may prevent water-imbibed seeds from expanding and bursting the tissues before after-ripening is accomplished, it takes little or no part in the dormancy of after-ripening of the seed. He was unable to force the germination of non-after-ripened *Juniperus* seeds by high temperature, alternate temperatures, wounding, warm bath, dry air, removal of coats; or by treatment with hydrogen peroxide, mercuric chloride, ether, carbon dioxide, oxygen, light, soil, dilute acids, dilute bases, nitrates, sulphates, or strong acids. Freezing and thawing as such have no forcing action on germination of the *Juniperus* seeds, neither do they hasten after-ripening. They bring about chemical changes in the seed, but these changes are different from those occurring during after-ripening. When seeds are about ready to germinate, PACK found that they are very sensitive and are killed by exposure to 5° C. The *Juniperus* seed has a dormant embryo that must be after-ripened before germination.

Mrs. DAVIS (4) found the sterilization of the naked seeds of *Cornus florida* difficult, due to the fact that the inner testa is very thin or the endosperm rich in food and the seeds are frequently infested with molds. She concludes that the pericarp, the outer testa, and the inner testa take no part in causing the delay; the moisture

intake with the pericarp and testas intact is as high as in seeds with these removed. After-ripened seeds break the pericarp readily. Delay is not caused by an immature embryo, as it is well differentiated several weeks prior to shedding. After-ripening of the dormant seed is favored by low temperatures, 0–5° C.

Rubus seeds vary according to species in the time required to weaken the coat with sulphuric acid. After the carbonized coats have been removed from the seeds they must be carefully sterilized, since treated seeds are very susceptible to molds. The delay in *Sphaeralcea remota* is due to the impermeable cuticle forming the outer layer of the seed coat. After this coat has been subjected to a 2–3 hour treatment with sulphuric acid it becomes permeable. Chipping slightly helps, as this method makes it possible for water to enter the seed. Mrs. DAVIS found that untreated seeds failed to swell or germinate, but that may have been due to the fact that the seeds were gathered late in the season. Treated seeds germinated and grew for a time in distilled water, and all seedlings showed remarkable vigor.

CROCKER (3) claims that in *Xanthium canadense* delayed germination is generally due to the seed coat rather than to the embryo. In the upper cockle-bur seed the delay is due to the exclusion of oxygen by the seed coat. No germination appeared in *Iris* seeds because the cap and endosperm stopped the absorption of water before the needed amount was obtained by the embryo. Those seed coats which exclude water are better for causing delay than those which exclude oxygen, because there is less respiration. The length of delay is due in nature to the persistence of the seed coats. In the case of *Xanthium*, the bur helps in causing the upper seed to germinate later. Seed coats reduce the oxygen supply, especially in the upper seed. High temperature brings about the germination of the upper seeds with the coats intact by raising the respiration ratio, which increases the rate of diffusion of oxygen through the seed coat.

DUVEL (5) has discovered that the factors affecting the vitality of the seed are maturity, weather conditions at time of harvesting, methods of harvesting, and curing. Immature seeds sown soon after gathering usually germinate readily, but if they are stored they soon lose their vitality. Seeds which are harvested in damp rainy weather are much weaker in vitality. By special care the life of the seed once

injured may be prolonged. Since moisture affects the longevity of seeds, they must be kept in a dry place where the temperature is low. DUVEL found that seeds treated in a sulphuric bath or in a vacuum usually showed delayed germination because the seed coat has hardened. In order to keep the vitality of the seed, it is better to have as little respiration as possible. Respiration brings about a chemical activity in the cells, which causes energy to be transformed, resulting eventually in the death of the seed. Respiration in the light is the same as in the dark if moisture and temperature conditions are the same.

FAWCETT (8) worked with 92 samples of weed seeds, representing 52 species. The seeds were collected in September, October, and November. Fifty seeds of each sample were placed in sand in boxes under the benches in the greenhouse. Every month from November to May this was repeated. Samples were also placed in sacks inside of a wooden box and a thin layer of sand placed around them. The boxes were then sunk in the ground so that just the top was exposed. Comparisons were made with the samples kept indoors. In April both lots were planted outdoors. FAWCETT concludes that weed seeds with thick seed coats require a more or less extended period of rest after maturity. Mustard and pepper grass seeds require little time for rest. Drying out weakens the vitality of nearly all weed seeds, and exposure to the natural periods for best seed germination, fall and spring, increases the power of germination.

HIMMEL (11) also believes that low percentage of germination for honey locust is due to the seed coats, for when the seeds had been treated with concentrated sulphuric acid the germination percentage increased very much. The older the wheat seeds the lower the germination percentage, but *Amaranthus* seeds displayed greater germination in the older seeds. The life of a dandelion seed is less than 8 years, but even the old seeds show good catalase activity. *Typha* seeds germinate better if they are pricked. Only very dilute acids and bases have any forcing effect on *Typha* seeds.

Miss EVANS (7) found that in after-ripened seeds with coats untreated, the restricting effect of the coats showed particularly at low temperatures 8–10° and 11.6° C., and again at high temperatures, 42° for Washington seeds and 46.1° for Indiana seeds. In both

cases these effects can be lessened by treating the coats with H_2SO_4, or abrading them with sand.

ATWOOD (1) found that there was less delay in germination in *Avena fatua* after the shell coats had been removed. Restriction of the oxygen supply by the seed coat acts as a limiting factor in germination. These seeds do not seem to be affected by light during germination. ATWOOD concludes that after-ripening occurs with the drying of the seed, but independent of the water content, as air-dried seeds soon after harvest yield lower germinative percentages than seeds of similar moisture content the following spring. Exclusion of water by the true seed coat does not explain after-ripening according to ATWOOD.

Material and methods

The primary purpose of this investigation was to discover the percentage of germination of the 1926 seeds of the following grasses:

1. *Hilaria mutica*
2. *H. mutica*
3. *Muhlenbergia porteri*
4. *Sporobolus airoides*
5. *Aristida purpurea*
6. *Scleropogon brevifolius*
7. *Bouteloua eriopoda*
8. *Muhlenbergia arenicola*
9. *Sporobolus cryptandrus*
10. *Bouteloua gracilis*
11. *Bouteloua eriopoda*
12. *Sporobolus giganteus*
13. *Aristida longiseta*
14. *Sporobolus flexuosus*
15. *Aristida longiseta*
16. *Bouteloua curtipendula*
17. *Sporobolus auriculatus*

Studies of these grasses have been made in previous years at the government laboratories in Washington, D.C., but the 1926 seeds were sent to the Hull Laboratory of the University of Chicago for experimentation by the writer. The seeds were collected by R. S. CAMPBELL on the Jornada Range Reserve Station at Las Cruces, New Mexico, and through him the following seeds from 1925 were obtained:

20. *Sporobolus airoides*
21. *S. auriculatus*
22. *S. cryptandrus*
23. *S. flexuosus*
24. *Epicampes emerslyei*
25. *Muhlenbergia arenicola*
26. *Panicum obtusum*

The seeds were still in the glumes on the spikes, hence it was necessary to separate not only the florets from the spikelets, but also

to remove the palea and lemma from the seeds. The seeds for the tests were selected at random, so that the results would be as representative as possible. Since some of the samples contained relatively few seeds, it was found necessary to use smaller amounts of seeds for some tests. In all cases the seeds were soaked for one half-hour in a 0.25 per cent solution of uspulun and then rinsed (washed) in distilled water twice for 15 minute periods. The blotting paper was also treated with the uspulun solution, and the Petri dishes containing cotton and filter paper were sterilized by autoclaving. These precautions were taken to insure the seeds against black mold attack. Only in a few instances were the seeds attacked by molds.

All seeds were tested at 25° C. in the moist chamber germinating oven in blotters and in Petri dishes. Those which did not do so well at 25° were tried at 35° C. Each species was tested for 100 per cent germination. In the preliminary tests, 10 seeds of each species were used. In some instances the lemma and the palea were not removed in the first tests, hence it was not certain that there were seeds present. Afterward it was made a point that all protective bracts be removed from the seeds. Exceptions were made in the case of the *Bouteloua* grasses, in which the writer could find no seeds; hence the entire florets were used in hopes that some might contain seeds.

The *Aristida* seeds were tested for germination in the light, as they were supposed to show better germination results there than in the dark. The *Sporobolus* seeds were treated in several ways. The seed coats of nos. 9, 12, 14 were pricked, and the seeds shaken for 4, 6, and 9 hours in bottles containing coarse white sand, in order to injure the seed coats, thus hastening germination through the more easy entrance of water into the seed. Some of the seeds of nos. 9, 12, 14, which had been shaken for various lengths of time, were planted in sandy loam and kept at room temperature to see whether they would germinate more readily after having their seed coats bruised. Nos. 9, 12, 14, 22, 23 were soaked in distilled water for a period of 4 days and then for one of 9 days. These seeds were then placed in the 5° C. oven for 7 days and 21 days, and then in the 25° C. germinating oven. Nos. 4 and 20 were treated with varying solutions of $CaCO_3$. Ten seeds of all the grasses, excepting the *Bouteloua* species, were planted in sandy loam.

TABLE I A

TABULAR DESCRIPTION OF SEEDS COLLECTED BY R. S. CAMPBELL

SEED NO.	SCIENTIFIC NAME	COMMON NAME	GEOGRAPHIC RANGE	PLACE OF COLLECTION	DATE OF COLLECTION (1926)
1.....	Hilaria mutica	Tabosa grass	Western Texas to southern Arizona and adjacent Mexico	1½ m. southwest of Red Lake Well	Sept. 19
2.....	Hilaria mutica	Tabosa grass	Western Texas to southern Arizona and adjacent Mexico	1 m. south of Middle Well	Sept. 1
3.....	Muhlenbergia porteri	Porter's bush grama	Colorado and western Texas to California and Mexico	2 m. south of Taylor Well	.....
4.....	Sporobolus airoides	Alkali sacaton	Washington and Nebraska to California and New Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
5.....	Aristida purpurea	Purple three-awn	Southwestern U.S. to southern Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
6.....	Scleropogon brevifolius	Burro grass	Arizona and western Texas to Mexico and South America	¾ m. northeast of Hdqrs.	Aug. 31
7.....	Bouteloua eriopoda	Black grama grass	Arizona and western Texas to Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
8.....	Muhlenbergia arenicola	Ring muhlenbergia	Colorado and Kansas to Texas and New Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
9.....	Sporobolus cryptandrus	Sand grass	Washington and Maine to Arizona and Texas	1¾ m. northeast of Hdqrs.	Aug. 31
10.....	Bouteloua gracilis	Blue grama grass	Manitoba to Mexico, and even to South America	St. Nicholas Canyon enclosure	Sept. 1
11.....	Bouteloua eriopoda	Black grama grass	Arizona and western Texas to Mexico	Enclosure no. 10	Nov. 9
12.....	Sporobolus giganteus	Gigantic sand grass	Southern New Mexico	1¾ m. northeast of Hdqrs.	Sept. 19
13.....	Aristida longiseta	Red three-awn grass	Southwestern U.S. and New Mexico	Aristida enclosure	Sept. 16
14.....	Sporobolus flexuosus	Wide-panicled grass	Nevada to Texas and Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
15.....	Aristida longiseta	Red three-awn grass	Southwestern U.S. and northern Mexico	1¾ m. northeast of Hdqrs.	Aug. 31
16.....	Bouteloua curtipendula	Side oats grama grass	Canada to New Jersey, California, and Mexico	Lion Den Canyon	Sept. 1
17.....	Sporobolus auriculatus	Dwarf dropseed	Western Texas to southern New Mexico	¾ m. north of Hdqrs.	Aug. 31

17.....	Sporobolus auriculatus	Dwarf dropseed	Western Texas to southern New Mexico	¾ m. north of Hdqrs.		Aug. 31

TABLE IB

TABULAR DESCRIPTION OF SEEDS

SEED NO.	SCIENTIFIC NAME	ALTITUDE OF PLACE OF COLLECTION (FEET)	TYPE OF SOIL	RAINFALL				PERCENTAGE DISSEMINATION	PERCENTAGE GERMINATION
				SEASONAL		ANNUAL			
				Inches	Zone	Inches	Zone		
1.....	Hilaria mutica	4200	Heavy clay	4.82	1	14.67	1	.....	34
2.....	Hilaria mutica	4200	Low swag heavy clay	7.51	4	18.29	5	.....	91
3.....	Muhlenbergia porteri	4050	Gravelly clay-sand	10.24	6	19.48	5	.....	75
4.....	Sporobolus airoides	4200	Clay loam	9.64	6	17.42	4	.....	92
5.....	Aristida purpurea	4200	Sandy loam	9.64	6	17.42	4	.....	60
6.....	Scleropogon brevifolius	4200	Clay loam	8.53	5	17.42	4	5	46
7.....	Bouteloua eriopoda	4200	Sandy loam	9.64	6	17.42	4	.....	.....
8.....	Muhlenbergia arenicola	4200	Clay loam	9.64	6	17.42	4	.....	60
9.....	Sporobolus cryptandrus	4200	Sandy loam	9.64	6	17.42	4	.....	75
10.....	Bouteloua gracilis	5600	Gravelly loam	11.23	8	21.17	7	5	.....
11.....	Bouteloua eriopoda	4050	Gravelly loam	7.65	4	17.42	4	.....	.....
12.....	Sporobolus giganteus	4200	Sandy loam	9.64	6	17.42	4	.....	97
13.....	Aristida longiseta	4300	Very sandy loam	5.73	2	18.53	2	25	17
14.....	Sporobolus flexuosus	4200	Sandy loam	9.64	6	17.42	4	30	42
15.....	Aristida longiseta	4200	Sandy loam	9.64	6	17.42	4	.....	53
16.....	Bouteloua curtipendula	4800	Gravelly loam	11.23	7	21.17	6	10	98.6
17.....	Sporobolus auriculatus	4100	Clay loam	8.53	5	17.42	4	.....	100

TABLE II

PRELIMINARY TEST ON BLOTTING PAPER, 25° C.; JANUARY 19–FEBRUARY 18, 1927 (31 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	10	1/27	10
2	Hilaria mutica	10	1/27	60
3	Muhlenbergia porteri	10	1/27	10
4	Sporobolus airoides	10	1/24	40
5	Aristida purpurea	9	1/24	33·3
6	Scleropogan brevifolius	10	2/3	20
7	Bouteloua eriopoda	10*	.....	.....
8	Muhlenbergia arenicola	10	2/3	30
9	Sporobolus cryptandrus	10	2/10	30
10	Bouteloua gracilis	10*	.....	.....
11	Bouteloua eriopoda	10*	.....	.....
12	Sporobolus giganteus	10	2/10	50
13	Aristida longiseta	10	.....	Moldy
14	Sporobolus flexuosus	10	2/15	80
15	Aristida longiseta	10	1/24	50
16	Bouteloua curtipendula	10	.....	Moldy
17	Sporobolus auriculatus	10	1/27	20

* Empty lemmas.

TABLE III

PRELIMINARY TESTS ON BLOTTING PAPER, 25° C.; FEBRUARY 4–25, 1927 (22 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	10	2/7	20
2	Hilaria mutica	10	2/7	100
3	Muhlenbergia porteri	10	2/7	80
4	Sporobolus airoides	10	2/15	20
5	Aristida purpurea	10	2/10	70
6	Scleropogon brevifolius	10	2/25	10 (9 moldy)
7	Bouteloua eriopoda	10*	.....	.....
8	Muhlenbergia arenicola	10	2/10	30
9	Sporobolus cryptandrus	10	2/15	60
10	Bouteloua gracilis	10*	.....	.....
11	Bouteloua eriopoda	10*	.....	.....
12	Sporobolus giganteus	10	2/25	40
13	Aristida longiseta	10	2/7	30
14	Sporobolus flexuosus	10	2/25	30
15	Aristida longiseta	10	2/7	40
16	Bouteloua curtipendula	10	2/7	100
17	Sporobolus auriculatus	10	2/7	20

* Empty lemmas.

TABLE IV

PRELIMINARY TEST IN PETRI DISH, 25° C.; JANUARY 27–FEBRUARY 25 (30 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	10	2/1	40
2	Hilaria mutica	10	1/29	100
3	Muhlenbergia porteri	10	2/1	60
4	Sporobolus airoides	10	1/29	70
5	Aristida purpurea	10	1/29	80
6	Scleropogon brevifolius	10	.....	Moldy
7	Bouteloua eriopoda	10*	.....	.....
8	Muhlenbergia arenicola	10	2/1	40
9	Sporobolus cryptandrus	10	1/22	40
10	Bouteloua gracilis	10*	.....	.....
11	Bouteloua eriopoda	10*	.....	.....
12	Sporobolus giganteus	10	1/22	40
13	Aristida longiseta	10	2/1	10
14	Sporobolus flexuosus	10	2/22	70
15	Aristida longiseta	10	2/1	80
16	Bouteloua curtipendula	10	2/3	100
17	Sporobolus auriculatus	9	2/1	100

* Empty lemmas.

TABLE V

PRELIMINARY TEST IN PETRI DISH, 35° C.; FEBRUARY 2–16 (14 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	10	2/7	40
2	Hilaria mutica	Not tried	.....	.....
3	Muhlenbergia porteri	Not tried	.....	.....
4	Sporobolus airoides	Not tried	.....	.....
5	Aristida purpurea	Not tried	.....	.....
6	Scleropogon brevifolius	10	.....	0
7	Bouteloua eriopoda	10*	.....	0
8	Muhlenbergia arenicola	5	.....	0
9	Sporobolus cryptandrus	10	.....	0
10	Bouteloua gracilis	10*	.....	0
11	Bouteloua eriopoda	10*	.....	0
12	Sporobolus giganteus	10	.....	0
13	Aristida longiseta	10	2/7	10
14	Sporobolus flexuosus	10	.....	0
15	Aristida longiseta	Not tried	.....	.....
16	Bouteloua curtipendula	10	2/7	100
17	Sporobolus auriculatus	Not tried	.....	.....

* Empty lemmas.

TABLE VI

PRELIMINARY TEST IN PETRI DISH, 25° C.; FEBRUARY 12–MARCH 15 (32 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	10	2/15	20
2	Hilaria mutica	10	2/15	80
3	Muhlenbergia porteri	10	2/15	10
4	Sporobolus airoides	10	2/17	90
5	Aristida purpurea	10	2/15	40
6	Scleropogon brevifolius	10	2/15	40
7	Bouteloua eriopoda	10*	.....	.....
8	Muhlenbergia arenicola	4	2/15	50
9	Sporobolus cryptandrus	10	2/15	100
10	Bouteloua gracilis	10*	.....	.....
11	Bouteloua eriopoda	10*	.....	.....
12	Sporobolus giganteus	10	2/15	100
13	Aristida longiseta	10	2/15	10
14	Sporobolus flexuosus	10	2/15	60
15	Aristida longiseta	10	2/15	60
16	Bouteloua curtipendula	10	2/15	100
17	Sporobolus auriculatus	9	2/15	33.3

* Empty lemmas.

TABLE VII

100 PER CENT GERMINATION TEST IN PETRI DISH, 25° C.; FEBRUARY 21–MARCH 16 (24 DAYS)

No.	NAME	NO. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
1	Hilaria mutica	50	2/28	34
2	Hilaria mutica	100	2/25	91
3	Muhlenbergia porteri	20	2/28	75
4	Sporobolus airoides	100	2/25	92
5	Aristida purpurea	100	2/25	60
6	Scleropogon brevifolius	50	2/28	48
7	Bouteloua eriopoda	100*	.....	.....
8	Muhlenbergia arenicola	20	2/25	60
9	Sporobolus cryptandrus	100	2/25	75
10	Bouteloua gracilis	100*	.....	.....
11	Bouteloua eriopoda	100*	.....	.....
12	Sporobolus giganteus	100	2/25	97
13	Aristida longiseta	100	2/25	17
14	Sporobolus flexuosus	100	2/25	42
15	Aristida longiseta	100	2/25	53
16	Bouteloua curtipendula	75	2/28	98.6
17	Sporobolus auriculatus	25	2/28	100

* Empty lemmas.

TABLE VIII

PRELIMINARY TEST ON BLOTTING PAPER, 25° C.; JANUARY 19–FEBRUARY 18 (31 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20	Sporobolus airoides	10	1/29	100
21	Sporobolus auriculatus	10*	.....	.....
22	Sporobolus cryptandrus	10	2/10	80
23	Sporobolus flexuosus	10	2/10	60
24	Epicampes emersleyi	10*	.....	.....
25	Muhlenbergia arenicola	10	2/1	30

* Empty lemmas.

TABLE IX

PRELIMINARY TEST IN PETRI DISH, 25° C.; JANUARY 27–FEBRUARY 25 (30 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20	Sporobolus airoides	10	2/1	100
21	Sporobolus auriculatus	Empty lemmas	.....	.....
22	Sporobolus cryptandrus	10	2/22	70
23	Sporobolus flexuosus	10	2/22	80
24	Epicampes emersleyi	Empty lemmas	.....	.....
25	Muhlenbergia arenicola	10	2/1	40
26	Panicum obtusum	10	2/7	30

TABLE X

PRELIMINARY TEST IN PETRI DISH, 35° C.; FEBRUARY 2–16 (14 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20	Sporobolus airoides	Not tried	.....	.....
21	Sporobolus auriculatus	5	2/10	20
22	Sporobolus cryptandrus	10	.....	.....
23	Sporobolus flexuosus	10	.....	.....
24	Epicampes emersleyi	Empty lemmas	.....	.....
25	Muhlenbergia arenicola	9	2/7	77.8
26	Panicum obtusum	10	.....	.....

TABLE XI

100 PER CENT GERMINATION TEST IN PETRI DISH, 25° C.; FEBRUARY 21–MARCH 16 (24 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
20.....	Sporobolus airoides	100	2/25	92
21.....	Sporobolus auriculatus	6	2/25	100
22.....	Sporobolus cryptandrus	20	2/25	95
23.....	Sporobolus flexuosus	40	2/25	92.5
24.....	Epicampes emersleyi	Not tried	.....	.....
25.....	Muhlenbergia arenicola	40	2/25	90
26.....	Panicum obtusum	100	2/28	21

TABLE XII

GERMINATION IN LIGHT IN PETRI DISH, ROOM TEMPERATURE, ROOM LIGHT; MARCH 1–16 (16 DAYS)

No.	NAME	No. USED	DATE OF FIRST GERMINATION	FINAL GERMINATION PERCENTAGE
5.....	Aristida purpurea	10	3/5	40
13.....	Aristida longiseta	10	3/5	20
15.....	Aristida longiseta	10	3/5	80

TABLE XIII

SEEDS SOAKED IN DISTILLED H_2O 4 DAYS, THEN IN GERMINATING OVEN AT 25° C. FOR 2 WEEKS

No.	NAME	No. USED	GERMINATION DURING SOAKING	GERMINATION AFTER SOAKING	PERCENTAGE GERMINATION
9.....	Sporobolus cryptandrus	15	0	0	0
12.....	Sporobolus giganteus	15	0	0	6.6
14.....	Sporobolus flexuosus	15	0	0	0
20.....	Sporobolus airoides	40	34	0	85
22.....	Sporobolus giganteus	15	0	0	0
23.....	Sporobolus flexuosus	15	0	0	0

TABLE XIV

SEEDS SOAKED IN DISTILLED H_2O 10 DAYS, THEN IN GERMINATING OVEN AT 25° C. FOR 2 WEEKS

No.	NAME	No. USED	GERMINATION DURING SOAKING	GERMINATION AFTER SOAKING	PERCENTAGE GERMINATION
9.....	Sporobolus cryptandrus	15	0	2	13.3
12.....	Sporobolus giganteus	15	2	0	13.3
14.....	Sporobolus flexuosus	15	1	0	6.6
20.....	Sporobolus airoides	15	12	0	80
22.....	Sporobolus giganteus	15	0	1	6.6
23.....	Sporobolus flexuosus	15	1	0	6.6

TABLE XV

SEEDS SHAKEN IN COARSE WHITE SAND, THEN IN 25° C. GERMINATING OVEN FOR 2 WEEKS

No.	NAME	No. USED	No. OF HOURS SHAKEN	No. OF DAYS SOAKED	PERCENTAGE GERMINATION
9.....	Sporobolus cryptandrus	10	6	5	10
12.....	Sporobolus giganteus	10	6	5	10
14.....	Sporobolus flexuosus	10	6	5	10

TABLE XVI

SEEDS SHAKEN IN COARSE WHITE SAND, THEN PLACED IN SANDY LOAM

No.	NAME	No. USED	No. OF HOURS SHAKEN	PERCENTAGE GERMINATION	No. OF HOURS SHAKEN	PERCENTAGE GERMINATION
9.....	Sporobolus cryptandrus	10	6	0	9	0
12.....	Sporobolus giganteus	10	6	0	9	0
14.....	Sporobolus flexuosus	10	6	0	9	0

TABLE XVII

SEEDS IN GERMINATING OVEN AT 25° C. FOR 21 DAYS

No.	NAME (10 SEEDS OF EACH SPECIES USED)	PERCENTAGE GERMINATION
	Without shaking or soaking (control)	
9.....	Sporobolus cryptandrus	10
12.....	Sporobolus giganteus	20
14.....	Sporobolus flexuosus	10
	Shaken four hours	
9.....	Sporobolus cryptandrus	0
12.....	Sporobolus giganteus	0
14.....	Sporobolus flexuosus	0
	Shaken six hours	
9.....	Sporobolus cryptandrus	10
12.....	Sporobolus giganteus	10
14.....	Sporobolus flexuosus	10
	Shaken nine hours	
9.....	Sporobolus cryptandrus	10
12.....	Sporobolus giganteus	20
14.....	Sporobolus flexuosus	10

TABLE XVIII

No.	NAME	NO. USED	PERCENTAGE GERMINATION	
			5° C. oven 7 days	25° C. oven 21 days
9.....	Sporobolus cryptandrus	15	0	0
12.....	Sporobolus giganteus	15	13.3	20
14.....	Sporobolus flexuosus	15	0	0
22.....	Sporobolus cryptandrus	15	0	0
23.....	Sporobolus flexuosus	15	0	0

TABLE XIX

SEEDS TREATED WITH $CaCO_3$ SOLUTIONS INSTEAD OF DISTILLED H_2O

No.	NAME	No. used	PERCENTAGE GERMINATION			
			0.5 per cent $CaCO_3$	1 per cent $CaCO_3$	5 per cent $CaCO_3$	10 per cent $CaCO_3$
4....	Sporobolus airoides (1926)	10	30	70	0	0
20....	Sporobolus airoides (1925)	15	87	80	0	0

TABLE XX

NO SEED SELECTION MADE; GLUMES PLACED IN PETRI DISHES AND THEN IN 25° C. OVEN

No.	NAME	RESULTS
7.....	Bouteloua eriopoda	No signs of germination
10.....	Bouteloua gracilis	No signs of germination
11.....	Bouteloua eriopoda	No signs of germination

TABLE XXI

SEEDS PLANTED IN SANDY LOAM AND KEPT AT ROOM TEMPERATURE (70° F.) FEBRUARY 28, 1927

No.	NAME	No. USED	DATE OF FIRST PLANT
1.....	Hilaria mutica	10	.....
2.....	Hilaria mutica	10	3/16
3.....	Muhlenbergia porteri	10	.....
4.....	Sporobolus airoides	10	.....
5.....	Aristida purpurea	10	.....
6.....	Scleropogon brevifolius	6	5/4
8.....	Muhlenbergia arenicola	10	.....
9.....	Sporobolus cryptandrus	10	5/5
12.....	Sporobolus giganteus	10	4/13
13.....	Aristida longiseta	10	.....
14.....	Sporobolus flexuosus	10	4/14
15.....	Aristida longiseta	10	.....
16.....	Bouteloua curtipendula	10	.....
17.....	Sporobolus auriculatus	10	.....

TABLE XXII

PRECIPITATION JORNADA RANGE RESERVE, 1926

STATION	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	AUGUST	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER	SEASONAL	ANNUAL
Headquarters	0.49	0.05	1.40	0.48	2.43	0.06	4.95	0.38	3.20	2.32	0.04	1.62	8.53	17.42
Midwell	0.56	0.01	2.28	0.59	3.09	0.01	3.21	1.06	3.24	2.23	0.04	1.97	7.51	18.29
Red Lake	0.49	0.01	1.91	0.74	2.97	0.02	1.85	0.42	2.55	1.75	0.02	1.94	4.82	14.67
Road Tank	0.01	0.01	1.82	0.67	1.71	0.00	5.19	0.22	3.91	2.37	0.06	1.78	9.32	18.35
Ropes	0.84	0.12	2.65	0.88	2.71	0.40	2.52	1.09	4.11	2.36	0.00	2.00	7.72	19.68
St. Nicholas	0.90	0.10	2.02	0.53	1.64	0.03	6.07	1.20	3.95	2.67	0.02	2.03	11.23	21.17
South Well	0.53	0.00	1.57	0.91	2.09	T	4.01	0.25	3.48	2.61	0.01	1.84	7.74	17.30
Stuart Well	0.55	0.00	1.97	0.71	1.93	T	4.75	0.25	2.78	2.66	0.04	1.73	7.78	17.37
Ragged Well	0.43	0.00	1.96	1.00	2.21	0.09	6.30	1.16	2.78	2.24	0.00	1.31	10.24	19.48
West Well	0.55	0.00	1.66	0.71	1.98	T	2.48	1.66	4.81	2.53	0.12	2.03	8.95	19.53
Period Study	.....	.....	1.62	0.51	2.00	0.01	5.42	0.52	3.70	2.52	0.00	1.89	9.64	.....
Enclosure no. 1	.....	.....	1.51	0.68	2.45	0.00	4.00	0.25	3.64	2.35	0.00	2.05	7.89	.....
Enclosure no. 10	.....	.....	1.37	0.88	1.94	0.02	3.81	0.31	3.53	2.34	0.00	2.00	7.65	.....
Enclosure no. 2	.....	.....	.....	.....	.....	.....	2.11	0.47	4.16	2.43	0.01	1.78	6.74	.....
Aristida	.....	.....	.....	.....	.....	.....	2.77	0.42	2.54	0.89	0.79	2.01	5.73	.....
Brown Tank	.....	.....	.....	.....	.....	.....	5.76	0.04	3.21	2.30	0.03	1.36	9.01	.....
Sand Hills	.....	.....	.....	.....	.....	.....	5.24	0.39	3.15	2.09	0.00	1.98	8.68	.....
New Well	.....	.....	.....	.....	.....	.....	5.76	0.81	3.58	1.84	0.40	1.95	10.15	.....
Average	0.59	0.03	1.92	0.72	2.28	0.06	4.23	0.60	3.46	2.25	0.13	1.85	8.29	18.23

TABLE XXIII

COMPARISON OF SEASONAL RAINFALL FOR (A) HILARIA MUTICA NO. 1 AND NO. 2; (B) ARISTIDA LONGISETA NO. 13 AND NO. 15*

	PRECIPITATION (INCHES)		
(a) Hilaria mutica, no. 1		Hilaria mutica, no. 2	
July	1.85	July	3.21
August	0.42	August	1.06
September (19 days)	1.61		
Total	3.88	Total	4.27
(b) Aristida longiseta, no. 13		Aristida longiseta, no. 15	
July	2.77	July	5.42
August	0.42	August	0.52
September (16 days)	1.52		
Total	4.71	Total	5.94

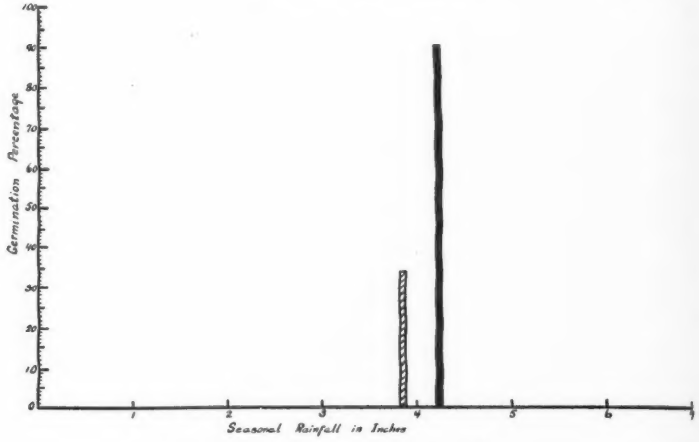
* See figs. 1, 2, 3.

TABLE XXIV

SEASONAL PRECIPITATION ON JORNADA RANGE RESERVE JULY, AUGUST, AND SEPTEMBER, 1926

No.	STATION	PRECIPITATION (INCHES)	RANGE (INCHES)
1	Red Lake	4.82	Under 5
2	Aristida Enclosure	5.73	5–6
3	Enclosure no. 2	6.74	6–7
4	Middle Well	7.51	7–8
5	Enclosure no. 10	7.65	7–8
6	Ropes	7.72	7–8
7	South Well	7.74	7–8
8	Stuart Well	7.78	7–8
9	Enclosure no. 1	7.89	7–8
10	Headquarters	8.53	8–9
11	Sand Hills	8.68	8–9
12	West Well	8.95	8–9
13	Brown Tank	9.01	9–10
14	Road Tank	9.32	9–10
15	Period Study	9.64	9–10
16	New Well	10.15	10–11
17	Ragged Tank	10.24	10–11
18	St. Nicholas	11.23	Over 11

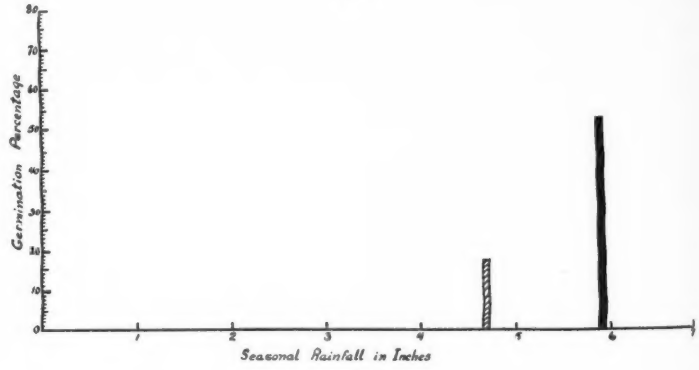
The areas allotted to the various stations are based on the records of the individual stations, supplemented by general observation throughout the year. Fig. 4 shows the approximate area covered by the different amounts of rainfall.



Comparison of Germination Percentages of
Hilaria mutica No. 1 and No. 2

Legend	Hilaria mutica No.1	Hilaria mutica No.2
	Seasonal rainfall 3.88 inches	Seasonal rainfall 4.27 inches
	Germination 34%	Germination 91%

FIG. 1



Comparison of Germination Percentages of
Aristida longiseta No. 13 and No. 15

Legend	Aristida longiseta No.13	Aristida longiseta No.15
	Seasonal rainfall 4.71 inches	Seasonal rainfall 5.94 inches
	Germination 19%	Germination 54%

FIG. 2

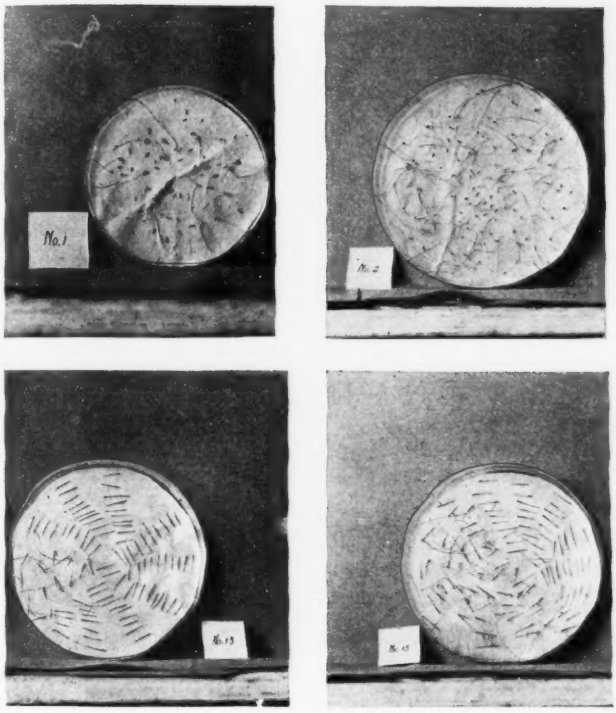


FIG. 3.—Upper row: comparison of germination of *Hilaria mutica* no. 1 (50 seeds, 34 per cent germination) and no. 2 (100 seeds, 91 per cent germination); lower row: comparison of germination of *Aristida longiseta* no. 13 (100 seeds, 17 per cent germination) and no. 15 (100 seeds, 54 per cent germination).

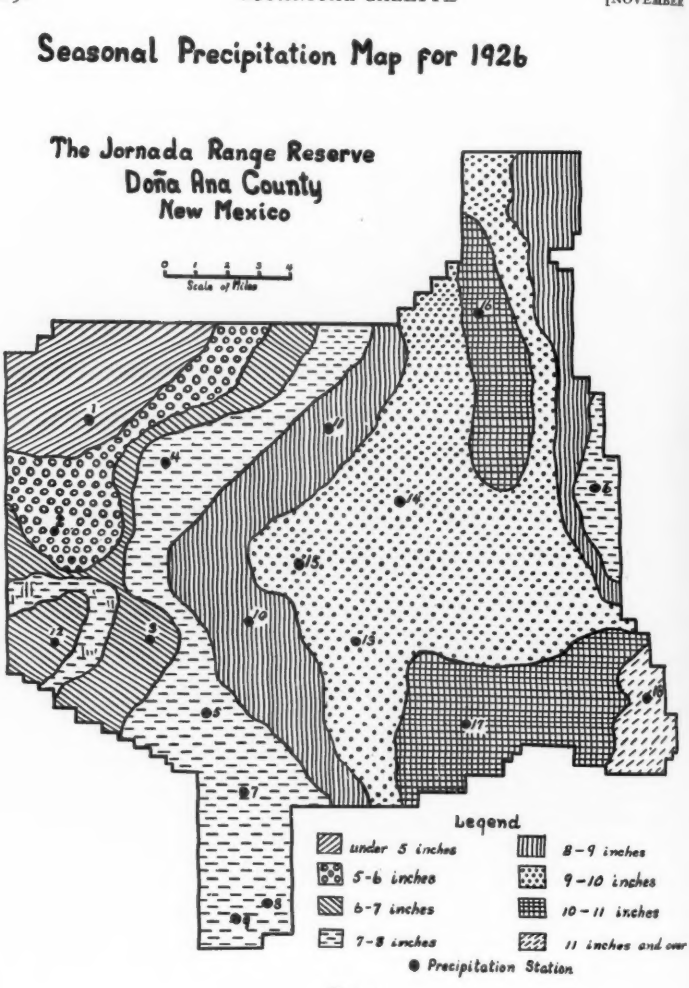

Seasonal Precipitation Map for 1926
The Jornada Range Reserve
Doña Ana County
New Mexico
Scale of Miles
Legend
under 5 inches
5-6 inches
6-7 inches
7-8 inches
8-9 inches
9-10 inches
10-11 inches
11 inches and over
Precipitation Station

FIG. 4

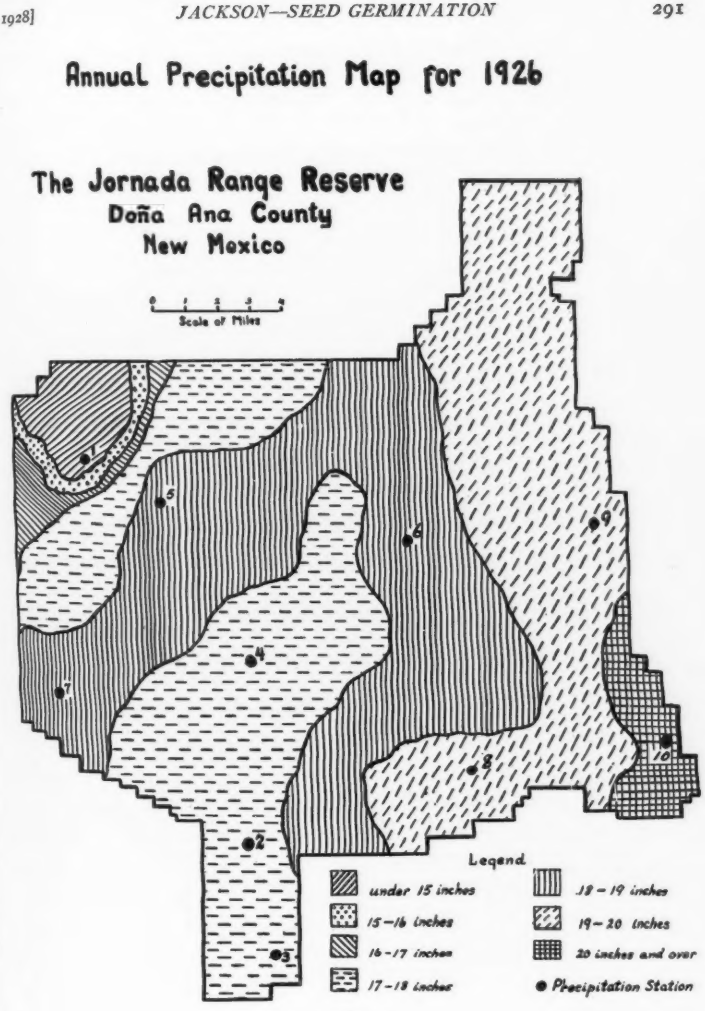

Annual Precipitation Map for 1926
The Jornada Range Reserve
Doña Ana County
New Mexico
Scale of Miles
Legend
under 15 inches
15–16 inches
16–17 inches
17–18 inches
18–19 inches
19–20 inches
20 inches and over
Precipitation Station

FIG. 5

The areas allotted to the various stations are based on the records of the individual stations, supplemented by general observation throughout the year. Fig. 5 shows the approximate areas covered by the different amounts of rainfall.

TABLE XXV

ANNUAL PRECIPITATION ON JORNADA RANGE RESERVE, 1926

No.	STATION	PRECIPITATION (INCHES)	RANGE (INCHES)
1	Red Lake	14.67	Under 15 15–16 16–17
2	South Well	17.30	17–18
3	Stuart Well	17.39	17–18
4	Headquarters	17.42	17–18
5	Middle Well	18.29	18–19
6	Road Tank	18.33	18–19
7	West Well	18.83	18–19
8	Ragged Tank	19.48	19–20
9	Ropes	19.68	19–20
10	St. Nicholas	21.17	Over 20

Discussion

From the tables of the various tests can be ascertained the germination percentages under different conditions. Table VII gives the final germination percentage for the seeds collected in 1926 and table IX for those gathered in 1925. From all the results it may be seen that the two lots of *Hilaria mutica* (nos. 1, 2) show a decided difference in their germination percentages. In no. 1 the germination percentage is always lower than in no. 2. By referring to the annual and seasonal rainfall figures, especially to the seasonal which includes just the rainfall during July, August, and September, when growth is taking place, one finds that no. 2 was collected in an area which received 2.69 inches more for the whole year than did the area in which no. 1 was growing. This probably accounts for the difference in the germination percentage, even though no. 2 was collected 19 days sooner than no. 1. The same is true of *Aristida longiseta* nos. 13 and 15. No. 15 was collected 16 days sooner than no. 13, but it gives much better germination results. The seeds of no. 15 received 1.23 inches more rainfall during the growing season than did the no. 13 seeds.

The *Bouteloua* (*B. eriopoda* and *B. gracilis*) had no seeds, so there was no germination percentage for either of them. Although tests were made in which the entire spikes were placed in sterilized Petri dishes in the 25° C. oven, no germination results were obtained. It is known that the *Bouteloua* grasses seed rarely, their means of propagation being vegetative rather than sexual.

Tables V and X show that germination is not increased by higher temperatures, as 35° C., nor does the alternation of low and high temperatures, that is from 5 to 25° C., increase germination (table XVIII). The best results are at 25 °C.

In regard to the *Sporobolus* seeds nos. 9, 12, 14, 22, and 23, which have an unusually hard seed coat, it was found necessary to prick them in order to obtain germination results. Shaking the seeds in bottles containing coarse sand helped germination somewhat, but the writer believes that much better results could be obtained if the period of shaking were considerably extended. This method would scratch the coats of the seeds in a way similar to that in nature.

Summary

1. The amount of rainfall during the year, especially during the growing season and at the time of harvesting, affects the vitality of the seed.

2. *Bouteloua eriopoda* and *B. gracilis* do not seed very often. Most of the florets are sterile, and because of their similarity to the fertile florets, are hard to distinguish from them.

3. *Aristida* seeds germinate just as well in the light as they do in the dark.

4. The seed coat is important in *Sporobolus* seeds, as it keeps out water and prevents germination. The seed coat must be punctured by some means before good germination results. Soaking affects the seed coats but little; shaking even for 9 hours in sand has little effect; and scratching or pricking hastens germination greatly.

5. The seeds of *Sporobolus airoides* do not need pricking to produce good germination results. The seed coat is more permeable to water than are the seed coats of the other species.

6. The *Sporobolus* seeds from 1925 retained their vitality very well.

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CHROMOSOME STUDIES IN THE CYPERACEAE, WITH SPECIAL REFERENCE TO SCIRPUS[1]

G. CLAUDE HICKS

(WITH PLATES X, XI)

Introduction

The Cyperaceae are of interest because they present situations unique among the Angiosperms. In the development of their microspores, the pollen mother cells undergo the usual meiotic divisions, but of the four nuclei so produced only one develops a grain. Another striking feature, that parallels the unique mode of formation of microspores, lies in the chromosome numbers investigated to date. Within the last decade, HEILBORN has published a series of papers (**18, 19, 20**) on the chromosomes, chromosome numbers, chromosome dimensions, and species formation, in the very large genus *Carex*. In the 44 species investigated, he found some 22 different numbers, ranging from 9 to 56, and which did not come under the law of multiples, namely, 9, 15, 16, 19, 24, 25, 27, 28, 29, 31, 33, 34, 35, 36, 37, 38, 40, 41, 42, and 56.

While this condition, termed aneuploid (TÄCKHOLM **42, 43**), also dysploid (JEFFREY **21**), is prevalent in genera of both plants and animals, HEILBORN maintains that *Carex* occupies a singular position in this regard. He finds that the differences of size in the chromosomes are not proportional, in the forms that do manifest multiples. He thus raises the question of quality and not quantity, and because the size classes are represented in quite different proportions in the different species, he concludes that polyploidy does not exist in *Carex*.

In explanation of such a condition, he gives in substance a summary (**19**) of the influences which seem to him to have borne and bear on the evolution of the chromosome numbers. They are: (1) the probable transverse division of original large chromosomes; (2)

[1] Contributions from the Laboratories of Plant Morphology, Harvard University.

a slow gradual change from the lower to the higher numbers; (3) this gradual change brought about through the duplication of entire chromosomes; (4) the duplication which is most probably a result of failing conjugation between homologous pairs, the univalents thus produced arranging themselves in the equatorial plate in the meiotic divisions and then being divided; (5) the cause of the lacking conjugation which is unknown, but may have been the result of previous crosses (although it is unlikely that crossing has played any great rôle in the evolution of the chromosome groups in this genus, as *Carex* hybrids are largely sterile); (6) the evolution proceeding from lower numbers to higher ones.

As regards species formation, HEILBORN finds that mutations of *Oenothera lata* type have been one of the most important processes, while hybridization can only be regarded as a factor rather inferior in importance.

In *Scirpus*, HAKANSSON (17) has reported the chromosome numbers 10, 13, 19, 21, 22, 23, 28, 29, 31, and 52. To explain this situation he suggests a transverse division of single chromosomes, and possibly a duplication of whole chromosomes, and is of the opinion that crossing may have been the cause of these numbers in some cases.

It accordingly seems profitable to study American material and other genera of the Cyperaceae for conditions, and thus to add to our cytological knowledge of chromosome number and behavior. The material which has been chosen would seem promising for this purpose, as it provides an excellent basis of comparison between stable and unstable species.

Materials and methods

The material was collected as it grew, and the environs of metropolitan Boston provided unparalleled advantages for the study. The region has been thoroughly botanized by sedge lovers, the stations have been recorded in *Rhodora* and in publications dealing with the flora of the region, and they are usually easily reached. The Gray Herbarium, too, provides unequaled facilities for information as to stations and for checking plant determinations.

The gathering of material is not without difficulties, however

in spite of these advantages, as some of the species are very closely allied and are almost exclusively determined by the characters of the mature fruit; consequently the taxonomic phase is an unusual one. This is especially true where the species and varieties are most closely related. In some cases, however, the plants are fairly definite, and one is greatly helped by the recorded flowering season and regional distribution.

Since it is highly important to have mature material with which to determine that which was gathered in a green condition, two lines of approach are open: (1) the collection of mature plants in the previous season, marking the spot and returning to collect at the proper time; or (2) the collection of immature plants in a place that can be marked, and returning to gather the material when it is ripe. It is of course very necessary to know the plants before going into the field, in order that there may be proper discrimination.

The inflorescences were collected on warm days and put immediately into Carnoy's fluid, since comparative experiment justified the use of that liquid. The air was then drawn quickly from the tissues by means of an exhaust pump, to insure rapid fixation. Material gathered in the early afternoon gave more satisfactory results on the whole. After being left in the fixative for 24 hours, the plants were washed in from two to four changes of 95 per cent alcohol.

If the plants were imbedded immediately they would not cut satisfactorily, because of the mineralization of the tissues. Moreover, the cytoplasm of water plants often contains dark substances which interfere with proper staining, so that it is generally necessary to demineralize and bleach. A stock solution for such is made by adding crystals of sodium chlorate to strong hydrofluoric acid, until a saturated solution is obtained. The material is passed into water, and is then put into specially prepared waxed bottles containing a diluted solution of the fluid described, and left for only such time as is essential to the necessary degree of softening and bleaching.

Following this treatment, the material is dehydrated and imbedded in nitrocellulose, in which distortion is much less likely than in paraffin. This is the method described by JEFFREY (21). The writer has also used with advantage the mass method, devised in connection with work on *Drosophila melanogaster* (JEFFREY and

HICKS 23, 24) and later improved. With this method exceptionally quick results are obtained satisfactorily. A Jung-Thoma sliding microtome was used for cutting sections. Both longitudinal and transverse sections were cut 10 and 5 μ thick. It was demonstrated that transverse sections were best for details.

Haidenhain's iron haematoxylin was used for staining and gave satisfactory results. Slow staining and slow decolorizing over long periods imparted to the chromosomes an unusually dark aspect. Further, when the cytoplasm was stained over night in a very weak solution of eosin in 30 per cent alcohol, most excellent results in contrast were obtained.

The sections were then mounted in Canada balsam, and after the benzol had evaporated the cover slips were pressed down with small lead weights.

In the study and drawing of the material, a Bausch and Lomb microscope, equipped with a 140° Abbé condenser, was used. Since strong light and resolution were very important factors, a 3 mm. 140° Bausch and Lomb apochromatic lens, together with a no. 15 "periplan" compensating ocular, provided an excellent combination of equipment. The drawings were outlined from typical stages of the pollen mother cells by means of a camera lucida with the tube length of the microscope at 160. They were then enlarged three times, except where noted, with the aid of a no. 346 Starrat rule graduated to fifths of a millimeter, in order that the proportions be maintained.

Counts of the chromosomes were made mostly from the heterotypic metaphase plates, but, where possible, use was made of the diakinesis and homotypic stages.

Observations

The chromosomes in some of the species are rather small, and it is difficult to know the exact conditions of affinity from a study of diakinesis. In many cases it seems best not to group the chromosomes into classes as no consistent results are obtained. Variations occurring in such cases may be due to the orientation of the chromosomes on the spindle. There is variation, however, among the chromosomes of each nucleus and between nuclei of the different species;

the *Scirpus lacustris* group has the largest chromosomes, while the *S. maritimus* group has the smallest. No successful attempts have been made to classify the chromosomes into different categories, as has been done in *Carex*. Haploid counts are recorded here.

Scirpus heterochaetus Chase.—Collections of this species were made along the Concord River at Bedford, and at Heard's Pond, Wayland, Massachusetts, and Cow Island Bay on the Charles River.

No early diakineses were seen. The size differences seen in the metaphase plates are not ordinarily evident. In fig. 1, 18 chromosomes are found as usual. These show size differences, one being conspicuously larger. In addition to the large chromosome, there are 6 of medium size and 11 of smaller size. The large chromosome may be quadrivalent, but it was not possible to demonstrate this condition. On one occasion there appeared to be 19 chromosomes.

The divisions are regular, excepting that the dark material (extranuclear chromatin) appears as it does in *S. validus*.

Mrs. CHASE (4), in defining the species, based its distinction on the three cleft styles, the triquetrous achene, fragile bristles, and glabrous scales. The solitary spikelets which she mentions are not always consistent features, however.

Scirpus acutus Muhl. f. *condensatus* (Farwell) Fern.—Mrs. CHASE comments that this species shows great variation regionally. A single collection proved to be this species, from an inspection of the material which was mature. This was gathered at the lower Mystic Lake. An unidentified collection from Cape Breton Island, Nova Scotia, showed the same results.

In the early stages of diakinesis the chromosomes are bivalent in character. In fig. 2 are 20 chromosomes lying in the metaphase plate. Of these one is conspicuously larger than the others. At times all the elements are round in form; at other times they show a constricted condition, and in this arrangement the large chromosome is quadripartite, a condition seen in late prophase also. It may be that this chromosome is quadrivalent.

The anaphase in the Mystic Lake material shows a lagging of the chromosomes, but no chromosomes are left in the cytoplasm. After this stage, the pollen mother cells are very much shrunken, and the condition persists through the homotypic division. Because of the

shrunken condition and the dark-staining capacity of the cells in this condition, the chromosomes cannot be followed any longer accurately.

The pollen is exceedingly bad, with disintegrating protoplasmic contents. The embryo sacs develop as usual.

Scirpus validus Vahl.—Individuals of this species were collected at Spot Pond, Nahant, Spy Pond, and on the Neponset River at Dedham Road Station, Massachusetts.

In diakinesis the nucleolus is rather large and is often constricted in outline rather than round. The chromosomes are to all appearances double, but at times perplexingly appear rounded off as single units.

The metaphase plates in all cases show 21 chromosomes (fig. 3); and although the chromosomes vary to some degree, there are no remarkable size differences, excepting that in Spot Pond material there is one large chromosome which is not always present in members of the same anther sac. They may be roughly classified as 7 of larger and 14 of smaller size.

The anaphases show themselves regular; in only one case was an irregularity seen. The chromosomes in going back to the poles do not manifest the paired condition. In the material from Spy Pond, some of the cells from the beginning of the telophases are very much collapsed.

In this species there is a vacuole in the apex of the pollen mother cell, and in this vacuole appears dark-staining material, such as is also found in the cytoplasm generally after the anaphase. KUWADA (28), describing such in *Zea mays*, writes of them as "extra-nuclear nucleoli." The same material appears in the cytoplasm before the anaphase, but at times it appears more pronounced after this stage.

The material persists throughout the homotypic divisions, which are quite regular. Often the perinuclear zones fuse and give an "8" effect. In interkinesis the chromosomes retain their form for some time. Cell plates sometimes appear as dark areas across the spindle fibers, and are not evident at times.

Scirpus atrovirens Muhl.—Only one collection proved to be of this species, and was made at Fresh Pond, Cambridge, Massachusetts, rather late in the season.

No suitable figures of diakinesis were found, and the metaphase plates were often not clear, due to clumping. In most cases a large double chromosome is present, with a second one at times. The number cannot be established definitely with the material at hand. Varying counts have been made, from 25 to 30 (fig. 4). This may be due to weakened affinity and may also be due to the fact that the chromosomes are hard to delimit.

Chromatin appears in the cytoplasm after the heterotypic, and for the most part the behavior of the chromosomes in the divisions is regular, with some very few cases of lagging. There are some cases, however, in which in the same anther sac are found heterotypic plates and telophases, interkinesis, and also homotypic metaphases. Cell plates are present in varying degrees.

One of the most prominent cases of cytomixis appears in this species (fig. 5). This phenomenon is seen in all stages, from synizesis to diakinesis, and renders accurate examination of the latter impossible.

The species has been found to develop green tufts of leaves from the axes of the inflorescence, after the achenes have begun to shed.

Scirpus georgianus Harper; *S. atrovirens* Muhl. var. *georgianus* (Harper) Fern.—Material of this variety was taken from two stations at Turtle Pond, Roxbury, one at Glacialis Pond, Cambridge, and one from Moose Hill, Sharon, Massachusetts.

All elements appear double in diakinesis, and never more than 28 were seen (fig. 6). The determination of the chromosome number is best accomplished in the metaphase plates, and this number has always been found to be the same. Cytomixis occurs up to the metaphase of the heterotypic divisions. The homotypic anaphases are perfectly regular. Faint and short-lived cell plates exist. In certain localities there are also found plants which have the tufts of leaves mentioned in connection with the preceding species.

Scirpus rubrotinctus Fern.—Material from Moncton, New Brunswick, merely provided examples of good pollen, while collections from Fresh Pond, Cambridge, and from Stoneham, Massachusetts, gave excellent cytological material for all stages desired.

Counts of diakinesis were not entirely satisfactory. There are al-

ways 33 chromosomes in the metaphase plates (fig. 7), 12 larger and 21 smaller, and the size differences can be recognized from a side view of the spindle.

Scirpus longii Fern.—After a great deal of searching for this species, a station was found in the Neponset River meadows at Dedham Road Station, Massachusetts.

This species does not appear in GRAY'S *Manual*, as it was not described until 1911 by FERNALD (7). Its nearest relatives are the *S. atrocinctus* group, and it is distinguished by the early season in which it flowers, its reddish brown achenes, and its sticky bracts. A cross-section through the stem at the bracts shows a deposit of material on the stem. The plants are robust, and the heads are larger than those of *S. atrocinctus* and present a shaggy appearance. The anthers are very long for the group in which the species belongs.

The late prophases were of no use, and the divisions of the pollen mother cells provided only a basis for the gross descriptions (fig. 8). They were absolutely useless in the determination of the chromosome number, as the chromosomes had clumped. By good chance a clear plate of the heterotypic division of the megaspore mother cell was discovered, and there are 34 chromosomes.

In the anaphases the chromosomes usually go back to the poles quite regularly, but there have been a few cases where they come to the metaphase plate irregularly (fig. 9). Much dark-staining material is found in the cytoplasm.

Scirpus atrocinctus Fern.—Material gathered at North Branch Station and Lakeville, New Brunswick, at Manchester, New Hampshire, and at Wenham, Massachusetts, shows identical conditions. The collection made at Wenham displays a brownish cast to the inflorescence, and at first sight would be taken for *S. pedicellatus*, but from the darkish cast of the involucels was determined as belonging to the species under consideration.

In diakinesis the chromosomes all appear normal. There is at times an irregular arrangement of the chromosomes as they come to the metaphase plate (fig. 10). The 34 chromosomes are divided into sizes: 6 larger, 18 medium, and 10 smaller. In no cases have quadrivalent chromosomes been found. Dark-staining material appears in the cytoplasm, as noted for the other species. The homo-

typic division is very regular, and the abortion of the nuclei is as usual.

Scirpus cyperinus (L.) Kunth. var. *pelius* Fern.—Members of this variety were collected at Mystic Lake, at Fresh Pond, Cambridge, and at Lake Waban, Wellesley, Massachusetts. The results were the same.

The stages in diakinesis are unsatisfactory as there was a great deal of clumping. A study of the metaphase plates showed 33 chromosomes. Size differences were also noted. All stages of divisions are regular (figs. 11, 12). The forma *condensatus* (Fern.) Blake of this variety is the same as the preceding one. The true species was found at Massapoag Lake, Sharon, Massachusetts, but after much cutting no results were obtained.

Scirpus americanus Pers.—Material was gathered at Heard's Pond, Wayland, Massachusetts, and the Charles River marsh, and on the marshes at Hyannis.

In the late prophases the elements seem to be in a paired condition. The metaphase plates show (fig. 13) 38 chromosomes of different sizes. All the stages are fairly regular, although there is frequently lack of uniformity of the chromosomes in the anaphase. The material requires much care to differentiate it properly, and at times the cytoplasm possesses a muddy appearance. Further, after the heterotypic divisions the cells are very much shrunken.

Scirpus olneyi Gray.—One collection was made at Hyannis, Massachusetts.

The chromosomes are to all appearances paired in diakinesis, and in the metaphase plates 39 chromosomes (fig. 14) are found. In the anaphase of the heterotypic division (fig. 15) are cell plates of extraordinary size. These likewise appear in the homotypic division. No irregularities are found in the cytoplasm, except that there is the same shrunken condition as noted in the preceding species.

Scirpus americanus Pers. (irregular form).—The material of *S. americanus* and of *S. olneyi* collected at Hyannis grew by a small pool of water at the foot of Garnold Street, and the stations of the two species merged into each other. In this middle region there were found specimens of *S. americanus* which were taller than, and

different from, the other members of the species, and the suspicion was held that the two species had crossed.

Fig. 16 shows the great range in size of the chromosomes found in diakinesis. Beyond this, these stages are of little value in providing information.

In the metaphase plates there is always a large number of chromosomes. Fig. 17 shows 64 elements, but the chromosomes may range from 50 up to that number. The difference in size is striking; 12 of the chromosomes are larger. From a side view the chromosomes are all split in the anaphase of the heterotypic division. This statement of the fate of the smaller chromosomes, which must be interpreted as univalents, is borne out by inspection of the profile view. All elements in the late anaphase show a twofold nature.

While there is great irregularity in the manner by which the chromosomes go to the poles (fig. 18), no cases have been found in which the chromosomes are left in the cytoplasm.

The homotypic divisions appear to be regular and all the chromosomes are split. In one case there were found 5 nuclei in the pollen mother cell, although no exact basis for this formation has been found. To establish this condition a careful checking is necessary, as the nuclei from a neighboring cell may be included.

Scirpus campestris Britton var. *fernaldi* (Bicknell) Bartlett.—Material of this variety from a single station in Cape Breton Island was kindly provided by Professor JEFFREY. The pollen mother cells need the utmost care in staining and differentiating, for the cytoplasm often possesses a bluish cast, despite the effort to have it clear and still retain the desired color in the chromosomes. The chromosomes are very small and numerous. In diakinesis there is dark-staining material in the cytoplasm. When the spindle is formed there are three poles at first, but this soon gives way to the bipolar condition.

The chromosomes are arranged very irregularly on the spindle and are impossible to delimit, but one can distinguish larger and smaller elements. The scattered condition persists even in late metaphase (fig. 19).

Fig. 20 represents the most peculiar condition of the anaphase. No earlier stages have been seen, but in the late condition a few

chromosomes are found on the spindle, while more occupy the poles. Surrounding the whole chromatin mass is apparently a membrane, and around the periphery chromatin masses are arranged. In the upper corner of the figure it would appear as if some of the chromatin were being extruded into the cytoplasm. In interkinesis (fig. 21) the daughter nuclei have reorganized and possess nucleoli. Between the nuclei appear chromosomes that have been left out in the cytoplasm, and in the cytoplasm generally dark-staining material is present. The homotypic division has always been found to be regular, but the elements are always clumped together.

In other cases the spindles of the heterotypic division possess regular plates, and there is present a number of chromosomes which, in the majority of countings, is very close to 55. Even in these regular divisions there is also dark-staining material in the cytoplasm. In the nuclear abortion, the dark elements described in the cytoplasm are in part included.

The pollen is largely imperfect, but in contrast to this condition, the embryo sac mother cells develop normally, so that crossing with other plants is possible. The cells of the megaspore mother cells are very large and are usually cut by the knife. After the heterotypic division in these, there is the same chromatoid material in the cytoplasm.

Scirpus campestris var. *paludosus* (A. Nelson) Fern.—The material of this variety came from stations on the Charles River marshes at Watertown, and from Hyannis, Nahant, and West Manchester, Massachusetts.

The pollen mother cells are much more regular in this variety than in the Cape Breton material. The plants from West Manchester are transitional between the varieties *paludosus* and *novae angliae*, in that they tended to an umbellate condition; but the cytological conditions are the same as those found in other stations.

In all the plants from the various stations, the dark-staining material seems to increase as meiosis progresses, and the vacuole in the apex becomes partly filled with it. The majority of counts average around 55, but as high as 56 and 57 have been made. The pollen in these is good and bad.

Scirpus fluviatilis (Torr.) Gray.—A single collection of this species was made at Heard's Pond, Wayland, Massachusetts.

A metaphase plate of the heterotypic division (fig. 22) shows 55 chromosomes. This number represents an average of many counts. Counts vary, due to the fact that the chromosomes come to the equator quite irregularly.

All stages of division have been found to be regular, and the size of the pollen mother cells is the largest of all the species investigated. While the divisions are regular, an increasing amount of dark-staining material is found in the cytoplasm as meiosis progresses, and as usual this material is surrounded by a clear area. After the divisions are over, the 4 main nuclei present an appearance different from the other "nuclei."

Scirpus robustus Pursh.—Two stations of this species have been investigated, one at Oak Island and the other at Neponset River Reservation, Milton, Massachusetts.

Material from both regions presents a muddy appearance in the cytoplasm, which renders differentiation a matter of great painstaking. Counts made in the heterotypic metaphase (fig. 23) plates range from 53 to 54 and 55 in equal numbers. The gross behavior of the chromosomes indicates no irregularity, save for the presence of the usual dark-staining material in the cytoplasm.

Exotic material of the *Scirpus maritimus* group (identified as *S. robustus* by COCKAYNE) was collected by JEFFREY in New Zealand. Counts of the metaphase plates vary exceedingly, as may be gathered from the condition depicted in fig. 24.

Scirpus caespitosus L., *S. planifolius* Muhl., and *S. hallii* Gray have also been investigated, and while no statement can be made on the chromosome numbers, the conditions of sporogeny are in line with the other material.

Discussion

The condition known as cytomixis is noticeable, very frequently, from the early stages of the prophase even up to the heterotypic metaphase. GREGORY (15) noticed it in sterile races of *Lathyrus odoratus*, and he describes his figures 16 and 17 as "the incomplete and abnormal division by constriction of the pollen mother cells, which occurs in a few cases." GATES (12) first coined this word for the

process of chromatin extrusion from the nucleus of one pollen mother cell into the cytoplasm of a neighboring one in *Oenothera gigas* and *O. biennis*, and it has been used ever since. He considered it a normal process, and apparently it causes no diminution in the chromosomes. ROSENBERG (34) records the occurrence in *Crepis* and *Drosera*, and ascribes it to faulty fixation. GATES and REES (13), working on pollen development in *Lactuca*, found this irregularity in all the species investigated, and stated that it was confined mostly to the early stages of the prophase, although there was one instance in the heterotypic anaphase. Miss YASUI (50), working on the cytology of some artificially produced hybrids, described in the prophase a passage of chromatin from one pollen mother cell through the pits of the membrane of the cell. SINOTO (40) made some observations on *Iris japonica* Thunb., and likewise deems cytomixis as due to poor fixation and mechanical injury. LONGLEY (32), writing of the dark material which he found in *Crataegus*, states: "I have seen what appeared to be an actual passing of this dark-staining through the walls between the pollen mother cell and tapetal cell."

In all the species of sedges wherein this odd occurrence is found in the prophase, the chromatin material makes its way from the nucleus out into the cytoplasm and passes over into the adjoining cell by spinning out into a fine thread. On entering the cytoplasm of a neighboring cell, the material rounds itself into a globular mass and creates a clear area around it. At times several such penetrating strands run across and coalesce. The result is not unlike a nucleus in appearance. When it occurs in diakinesis, the material passing over appears to be made up of chromosomes, the chromosome passing out into the cytoplasm and becoming a fine thread as it passes across. The same behavior appears where chromosomes pass over in the heterotypic metaphase.

The phenomenon is most difficult to explain, but it is to be observed in the present connection that the material was not handled roughly, and the best of care was taken in preservation. It is of further interest to note that the occurrence has taken place in almost all the common and standard fixatives: Flemming's strong solution, Bouin's, Benda's, Hermann's chromo-acetic, Carnoy's, Merkel's alcohol and acetic, both strong and medium.

Whether or not the dark-staining material appearing in the cytoplasm is in part the after effects of cytomixis cannot be decided. In the plants under consideration, the dark material where it occurs is first seen quite often in the middle and late stages of the prophase. In the narrow apices of the pollen mother cells, a vacuole is frequently found in radial aspect. Often in diakinesis these vacuoles are not filled with any material, but after the heterotypic division they become partly filled with dark material, staining the same as chromatin, and there is in addition more of the material to be found in the cytoplasm. After the homotypic there may be a further increase. Some of the material may be interpreted as being chromatin, as in the case of the mule.

The fate of the material has already been discussed. Some is included with the aborting nuclei, and the rest apparently disorganizes and disintegrates during the time that the exine and intine coats are laid down; for rarely, if at all, is there any of the material left in the pollen cell.

SHARP (**39**), in dealing with the term hybridization, is of the opinion that "the sexual union of any two protoplasms differing in hereditary potency, no matter how slight the difference, constitutes an act of hybridization in the fundamental sense." A survey of the literature on hybrid cytology shows that it is upon the degree of the difference of hereditary potency that the differences in behavior found in sporogeny depend.

This view is borne out by the results of GUYER (**16**), who crossed closely and more distantly related pigeons. He found that sterility increased in direct proportion as the parents were divergent from one another. The sterility was the result of abnormalities in meiosis; greater or less degeneration of the germ cells; and abnormalities in the structure of the spermatozoa, which showed great variation in size. He assigns the reason of sterility to the fact that there is lack of union in synapsis because of the incompatibility of the different plasmas.

SCHÜRHOFF (**38**) investigated a hybrid *Saxifraga* between *S. decipiens* and *S. granulata*. CORRENS had found the form occurring spontaneously, and later crossed the two parents to demonstrate the parentage of the hybrid. He found the meiotic divisions and the

pollen formation normal, and we must conclude that the parents were very closely related because of the manifested compatibility.

BLACKBURN and HARRISON (2) studied the two crosses *Salix viminalis*×*S. purpurea* and *S. caprea*×*S. lanata*. These two might have been taken as pure species, for there is regular pairing in all the different phases. The divisions are regular, and there is no slightest evidence of a hint at hybridity, for the pollen is good in addition. In the cross *S. aurita*×*S. phylicifolia* there were irregular heterotypic and homotypic divisions which resulted in small nuclei. Tetrads were always formed, however, but degenerated, resulting in sterile pollen.

In the case of the sterile hybrid *Ribes schneideri*, TISCHLER (45) shows in its mitosis no more irregularity than in *R. gordiana*, but the pollen mother cells are found in contrast to give rise to sterile gametes.

It is not without sufficient grounds, then, that the idea is put forth that we should not expect any great irregularities if hybridism occurs in some of the sedges investigated, especially when the species are so similar in the immature condition that the specific characters often cannot be distinguished. This last condition is particularly true of the huge genus *Carex*, investigated by HEILBORN. It is further a notable fact in many hybrid and apogamous plants that, while the pollen may become abortive, the megaspore mother cell is quite able to function and seeds may be developed; and since pollen formation in the sedges is analogous to the formation of the megaspore, and since there is only one chance in four to show any incompatibility, it may not be expected that any great irregularity manifests itself.

FOCKE (10) records *Syringa rothamogensis*, a cross between *S. vulgaris*×*S. persica*, on the basis of its completely sterile pollen and of its history as it arose under garden conditions. JUEL (26) sought the causes of sterility and found them in the abnormalities of tetrad formation. The chromosomes from the parents seemed to be incompatible in part, and the irregularities followed as a consequence. TISCHLER (45) examined the same form cytologically, and reported regular as well as irregular meiosis. He believes that other factors as well as hybridity can be the cause of irregularity. More recently,

Miss BORGENSTAM (3) has sought to demonstrate that lowering of the temperature causes the conditions as found by JUEL and TISCHLER.

A serious objection to Miss BORGENSTAM'S interpretation is that the form is of known hybrid origin, and more reliability could be placed on her results had a known homozygous form been chosen. Many hybrids have been described which have both irregular and regular divisions, and further hybrids often show more irregularity in the first generations than in those that follow.

It would seem that *Scirpus acutus* f. *congestus* is most probably of heterozygous origin, because of the condition of the microspores and on the basis of its regional variability. This view has long been held by JEFFREY (22, 23, 25) and by his students (FORSAITH 11, COLE 5, LONGLEY 31, 32).

The roses have also been distinguished by their polymorphy, and the cause was unknown or only suspected for some time. The work of TÄCKHOLM (42, 43) provides almost an epitome of our knowledge of hybrid cytology. He divides this great group into three great classes: (1) those characterized by the occurrence of only paired chromosomes; (2) the very polymorphic *Canina* section in which bivalents and univalents are found, usually in multiples of seven; (3) aneuploid forms in which bivalents and univalents do not form multiples of seven.

Many of the recorded hybrids of the first class are regular in their reproductive divisions and the formation of spores. The *Canina* groups are of special interest because they are analogous to the well known *Drosera* scheme of hybrids. By their crossing, the *Canina* groups have given rise to the aneuploid species. The numbers here do not form multiples, due to the fact that the pollen grains do not possess the full quota of chromosomes, because of the double splitting of the univalents as found in the irregular forms of *Scirpus americanus*, and because of the irregularities in distribution during division. These aneuploid numbers, due to the agency of hybridism, are of great importance in connection with the explanation of the origin of dysploidy in other plants. It will be seen that the roses are no exception in this regard.

GEERTS (14) crossed *O. lamarckiana*×*O. gigas*, and found as a result that there were 7 bivalents and 7 univalents. Chromosomes from the gemini go to form the main tetrad nuclei, while the univalent chromosomes go to form mainly dwarf nuclei. In the F_2 generation he found 14 chromosomes, pointing to the elimination of the chromosomes which failed to pair in diakinesis. Miss LUTZ (29) in the first generations of the cross *O. lata*×*O. gigas* found the following numbers: 15, 21, 22, 23, 29, and 30. KIHARA (27) has observed in the F_2, F_3, and F_4 plants of pentaploid *Triticum* hybrids the numbers 28, 31, 32, 37, 38, 39, 40, 41, and 42. This case is especially interesting, as the F_1 had 35. DE MOL (33) has found in cultivated races of *Hyacinthus* the following varying numbers: 19, 20, 21, 22, 23, 24, 27, 28, and 30.

TISCHLER (44) has studied a sterile *Bryonia* hybrid. In the prophase chromatin was to be found in the cytoplasm. Further, the chromosomes were arranged irregularly on the spindle and seldom form a regular plate. Likewise, the homotypic spindles were irregular, but supernumerary pollen grains were seldom found. The chromosomes not only lag but are also extruded into the cytoplasm. Later (48) he interpreted his findings as being due to crossing of species of unequal chromosome numbers.

In *Rubus* and *Crataegus* LONGLEY (31, 32) has found conditions which seem to explain polymorphy. He concludes that species have been modified by hybridization. In *Crataegus* he finds three classes: (1) diploid species in which pollen formation is normal; (2) triploid and tetraploid species that show irregularities in their chromosome distribution and are accompanied by polycary and polyspory; (3) triploid and tetraploid species that are unable to form pollen grains and are sterile, due to the fact that they are the products of distant crosses. He divides *Rubus* into two major classes: (1) diploid species in which meiosis and pollen formation are normal; (2) polyploid species which are triploid, tetraploid, pentaploid, hexaploid, and octoploid. These polyploid forms are characterized by irregularities in chromosome distribution which lead to polycary and polyspory.

Wheat has been the subject of much investigation. SAX (36, 37) divides the species on the basis of the gametophytic number: Ein-

korn 7, Emmer 14, and Vulgare 21. Crosses between the different sorts give rise to cytological behavior like that in *Drosera obovata*. He finds that sterility increases in proportion as the number of univalent chromosomes increases in the reduction divisions. The figures of the anaphase are not unlike those of the irregular variety of *Scirpus campestris* found in Cape Breton.

FARMER and DIGBY (6) investigated *Polypodium schneideri*, which arose as a cross between *P. aureum* and *P. vulgare* var. *elegantissimum*. The spores were incapable of germination. The haploid number in *P. aureum* is 34, while the corresponding number in the other parent is 90. The hybrid, however, possessed usually from 95 to 105 chromosomes, seen in the late prophase, and in a few cases more were found: an indication that the number of chromosomes which pair is by no means a fixed one. The mitoses are very irregular, and often regression changes develop in the spore mother cells, with the result that the tetrads are but rarely formed and soon degenerate.

WODSEDALEK (49) has investigated the causes of sterility in the mule. It is in many respects the zoological counterpart of the hybrid fern. The divisions in the spermatogonial cells are quite normal, but in the late prophase of the primary spermatocyte the pairing of the chromosomes is never complete. It is also inconsistent, as seen in the number of chromosomes present, which ranges from 34 to 49 as compared with the 51 chromosomes found in the spermatogonial cells. The abnormalities invariably occur in the metaphase of the primary spermatocytes, and there is evidence that the chromatin material is eliminated. The result is almost complete sterility. The work provides an absolute demonstration of the concomitancy of hybridity and sterility.

The Boston fern has long been known for its extreme variability, almost complete sterility, and great vegetative vigor. The causes of polymorphism in this plant have been described by JEFFREY and ROSCOE (25). The reduction divisions are quite abnormal, the sporangia are shrunken, and the spores are abortive in their development since they are shriveled and devoid of protoplasmic content. Accordingly, this so-called mutating plant is apparently to be regarded as a hybrid.

Conclusions

The evidence presented has emphasized the point of view that, not only has polyploidy originated through crossing, but aneuploidy as well finds its explanation in that phenomenon. The conditions that have been described, in a genus that is comparatively stable taxonomically, have provided significant suggestions toward explanation of non-multiploid polyploidy in the American species of *Scirpus* and in other groups, namely, as the result of crossing.

Scirpus campestris var. *fernaldi* shows abnormal meiotic divisions, and the definite cases of chromatin extrusion seen in the pollen mother cells seem to indicate that this form has arisen through crossing. Material from other stations of this species in Massachusetts shows fairly regular divisions but some sterility. The dark-staining material present in these forms, as well as in other species, leads to the suspicion that this chromatoid material may be further evidence of crossing.

Other definite cases where hybrids have apparently arisen are to be found in *Scirpus acutus* f. *congestus*, because of the manifested sterility and loose affinity of the chromosomes in meiosis. Further, the cytological irregularities of *S. americanus* at Hyannis, manifesting the failure of chromosomes to pair, seem to be clear indications of heterozygosis. The cytological conditions found here may also serve to explain the cause of great variation in size, shown by more regular forms of this species in different localities.

In view of the cytological evidence of hybridism here presented, it is to be concluded that *Scirpus* is apparently being modified by crossing, and it is highly probable that the same situation has also prevailed in the past.

An interesting question arises in regard to the conclusions reached by HEILBORN in *Carex*. As has been pointed out in this paper, HEILBORN comes to the conclusion that the non-multiploid (dysploid) polyploidy found in *Carex* is the result of mutation, and has no obvious connection with previous hybridism. It seems unlikely, in view of the conditions here recorded for *Scirpus*, that this point of view can be maintained.

Summary

1. Aneuploidy is present in American species of *Scirpus*.

2. The chromosome numbers found range as follows: 18, 20, 21, 25–30, 28, 33, 34, 38, 39, 50–64, 53–55, 55, 55–57.

3. Conditions similar to that found in known hybrids have been discovered.

4. The seemingly hybrid conditions are to be correlated with taxonomic variability and polymorphy.

5. The interpretations of HEILBORN cannot be applied to the species investigated.

6. Hybridization is offered as a probable explanation of the aneuploidy found.

The writer wishes to record here his indebtedness to Professor E. C. JEFFREY, at whose suggestion this research was undertaken. Appreciation is also expressed for the plant determination checks made by Professor M. L. FERNALD.

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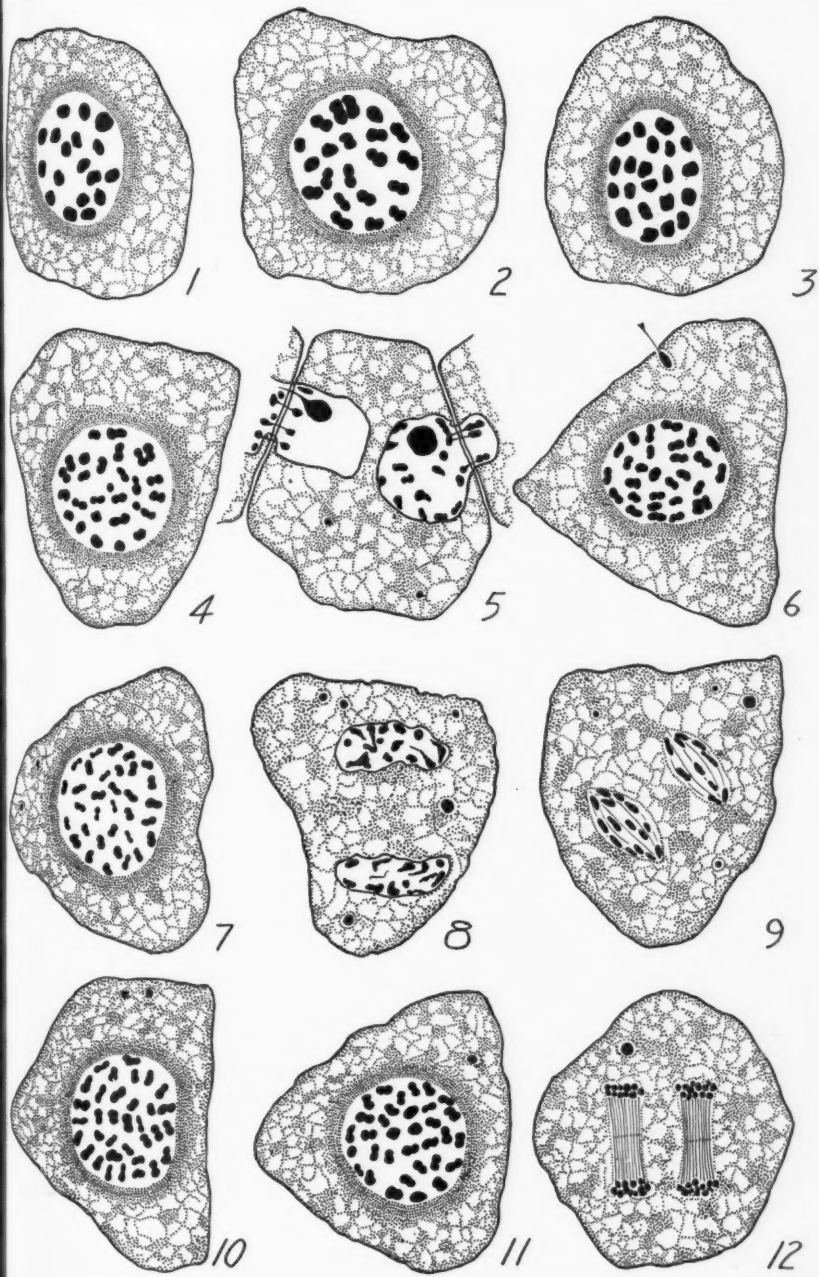
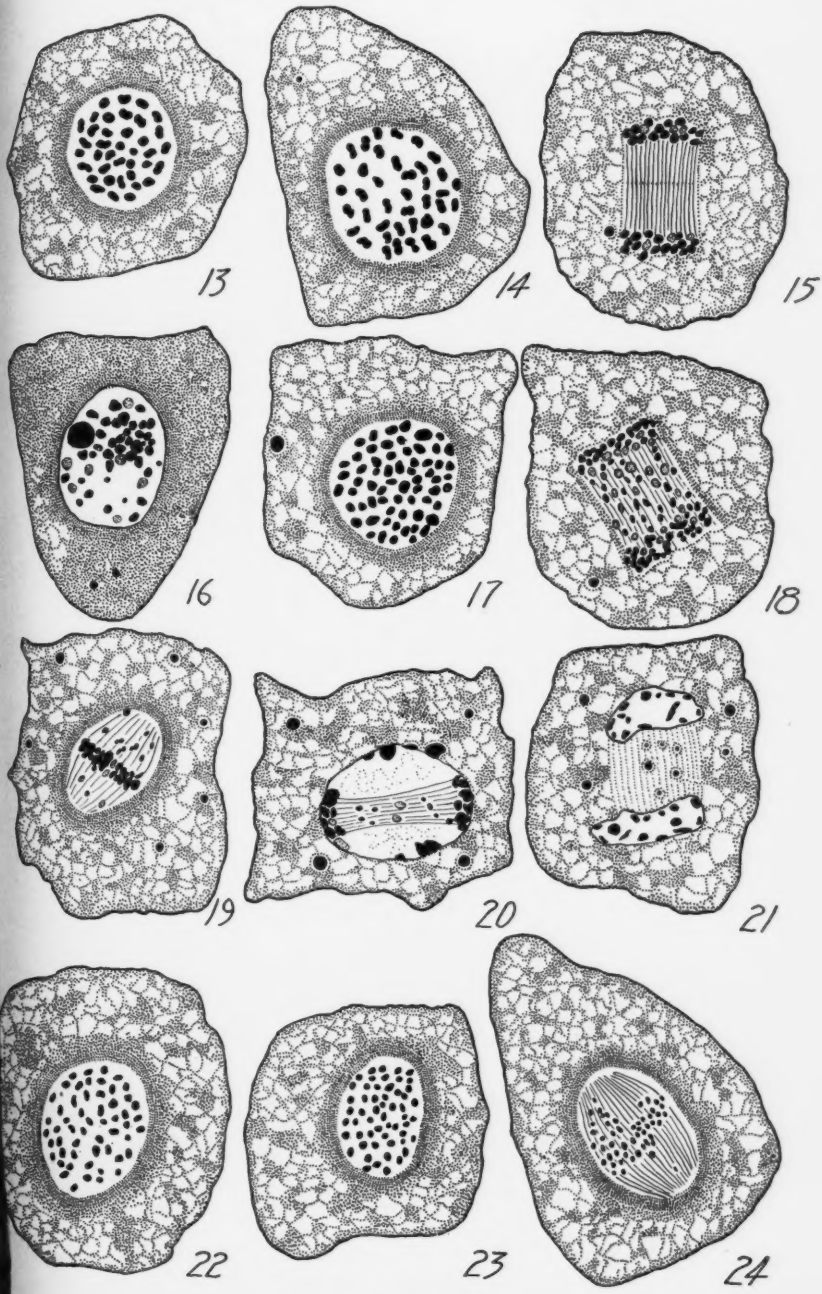


HICKS on SCIRPUS



HICKS on SCIRPUS

divis

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EXPLANATION OF PLATES X, XI

PLATE X

FIG. 1.—*Scirpus heterochaetus:* metaphase of first division, polar view.
FIG. 2.—*S. acutus* f. *congestus:* metaphase of first division, polar view.
FIG. 3.—*S. validus:* metaphase of first division, polar view.
FIG. 4.—*S. atrovirens:* transverse section, polar view of metaphase of first division.
FIG. 5.—Same: cytomixis.
FIG. 6.—*S. atrovirens* var. *georgianus:* polar view of metaphase of first division.
FIG. 7.—*S. rubrotinctus:* transverse section, polar view of metaphase plate.
FIG. 8.—*S. longii:* anaphase of first division, transverse section.
FIG. 9.—Same: early metaphase of second division.
FIG. 10.—*S. atrotinctus:* transverse section, polar view of metaphase plate.
FIG. 11.—*S. cyperinus* var. *pelius:* metaphase of first division, polar view.
FIG. 12.—Same: anaphase of second division showing cell plates.

PLATE XI

FIG. 13.—*S. americanus:* metaphase of first division, polar view, tangential section.
FIG. 14.—*S. olneyi:* metaphase of first division, polar view.
FIG. 15.—Same: anaphase of first division, tangential section.
FIG. 16.—*S. americanus:* irregular form, diakinesis.
FIG. 17.—Same: metaphase of first division, polar view.
FIG. 18.—Same: anaphase of first division, transverse section.
FIG. 19.—*S. campestris* var. *fernaldi:* diakinesis.
FIG. 20.—Same: anaphase of first division.
FIG. 21.—Same: telophase of first division.
FIG. 22.—*S. fluviatilis:* metaphase of first division.
FIG. 23.—*S. robustus:* metaphase of first division.
FIG. 24.—Same: New Zealand form, metaphase of first division.

ROOT INHERITANCE IN PEAS

F. C. JEAN

(WITH FOUR FIGURES)

Introduction

Since the time of MENDEL (5) it has been known that above-ground characters, such as tallness and dwarfness in peas, are transmissible from parent to offspring. Little or nothing, however, is known about the hereditary behavior of the roots. A review of the literature reveals not only that no previously recorded investigations as to the hereditary relations of pea roots have been made, but that the genetic behavior of roots in general is almost a wholly unexplored field.

HOWARD and HOWARD (4), investigating the fiber plant *Hibiscus sabdariffa*, found the tendency for the roots to discolor in wet seasons and an intolerance of constantly wet soil to be inherited. HOLBERT and KOEHLER (3), while investigating the extent and anchorage of corn root systems, discovered an apparent correlation between certain root characters and a susceptibility to leaf firing and root rot. Their data "suggest that the genetic factors responsible for the reduced root systems in the strains susceptible to root rot and leaf firing, respectively, are recessive." COTTLE (1) found the tendency to lodging in certain strains of corn to be due to a fragility of roots which was transmitted. VENKATRAMAN (7), making a study of sugar cane, felt that his observations indicated the inheritance of, and influence of the pollinating parent on "depth, penetration, resistance to water logging, and other characters of the root."

These statements are found, with the exception of VENKATRAMAN'S study, in the reports of research not primarily concerned with root inheritance. They are incidental only, and tend to show just how little definite work has been done in this field. This investigation was carried on in an attempt to determine whether root length is a genetic factor in peas, and whether it segregates in the F_2 generation as does the height of the top.

Material and method

Commercial varieties of Nott's Excelsior and of Telephone peas, both strains of *Pisum sativum*, were selected for this work. The first is a dwarf plant which, under field conditions, attains a height of about 1 ft. 8 in.; the latter reaches a height of approximately 4 ft., or more than twice that of the small plant.

In excavating the root systems, the trench method, as employed by WEAVER, JEAN, and CRIST (8), was used with slight modifications. This consisted in digging a trench of convenient size, about a foot from the base of the plants, to a depth well below the maximum root penetration. The roots were then dissected out of the soil with appropriate tools, measured, and sketched to scale. The root systems of ten plants in each parental and hybrid generation were examined.

TABLE I

VALUES OF TOP AND ROOT MEASUREMENTS AND THEIR RELATIONS (TOP MEASUREMENT FOR 20 PLANTS, ROOT MEASUREMENT FOR 10 PLANTS)

PLANTS	RANGE OF HEIGHT (IN.)	MEAN HEIGHT (IN.)	PERCENTAGE RELATION OF HEIGHT	RANGE OF MAXIMUM ROOT PENETRATION (IN.)	MEAN MAXIMUM ROOT PENETRATION (IN.)	PERCENTAGE RELATION OF ROOT PENETRATION
Parental Nott's Excelsior	13 to 27	19.2	100.0	22 to 34	28.2	100.0
Parental Telephone	36 to 54	46.5	242.2*	28 to 45	35.8	127.0
F_1 generation	36 to 48	41.1	214.1	31 to 49	37.9	134.4
Dwarf F_2 segregates	14 to 26	20.0	104.2	25 to 37	30.9	109.6
Tall F_2 segregates	38 to 56	44.9	233.9	30 to 50	40.9	145.0

*In computing the percentage relations for the mean height of tops and the mean depth of roots, the values for the parental Nott's Excelsior were taken as 100 per cent.

CULTURE AND ENVIRONMENTAL CONDITIONS

The original parents were grown in the greenhouse at the University of Nebraska during the winter of 1924–25. Stamen emasculation was made under a hand lens to insure that no self pollination occurred. Cross pollination was then accomplished by hand in the usual manner, and the flowers immediately bagged to prevent possible insect contamination. The F_1 generation was grown during the season of 1925 in a field near Greeley, and permitted to self fertilize.

In 1926 representatives of all generations, that is, both parental

lines and the F_1 and F_2 generations, were grown for the purpose of examination and comparison. The seeds were planted April 26 in separate, adjacent plots. The rows were 3 ft. apart, and the plants were spaced at intervals of 3 in. in the rows. Surface cultivation was given from time to time, to prevent the growth of weeds and to keep the soil in good tilth.

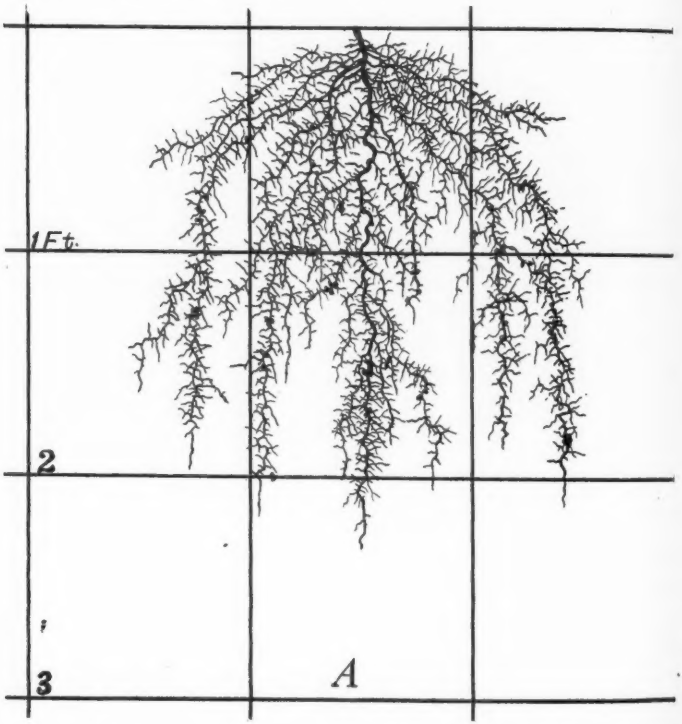

FIG. 1 *A*.—Dwarf pea root system: Nott's Excelsior, parental

The weather was characteristic of northern Colorado east of the Rockies. The precipitation from May 1 to August 1 was 5.7 inches, most of which came in light showers. The mean night temperature for the same period was 59° F. and the mean day temperature 76° F. The average night and day humidities were 89 and 48 per cent

respectively. Soil moisture determinations were made regularly, and when, on June 7 and 28, the soil became too dry for good growth the plots were irrigated.

Top and root characteristics

All plants were examined July 9 and 10. The parental Nott's Excelsior were practically mature. Some blossoms were still to be

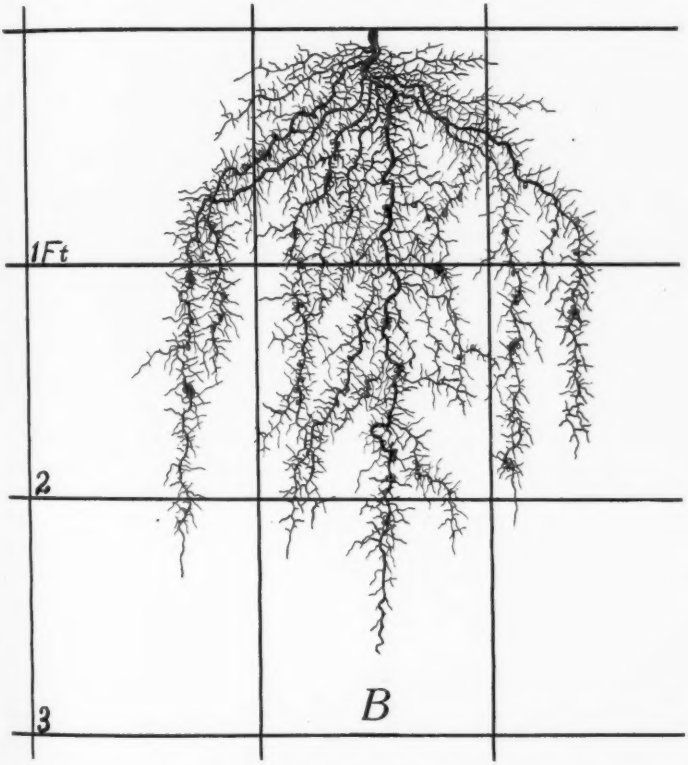

Fig. 1 *B*.—Dwarf pea root system: F_2 segregates

seen, but many of the fruit pods were so old as to be light colored and crinkled. The average height of twenty successive plants was 19.2 in. (table I).

The root system was characterized by a tap root which pursued a more or less vertical course downward through the soil. In three of the ten plants examined the tap root had died at 3–8 in. below the surface, its functions then being assumed by one or more of the larger laterals. The maximum depth of penetration ranged

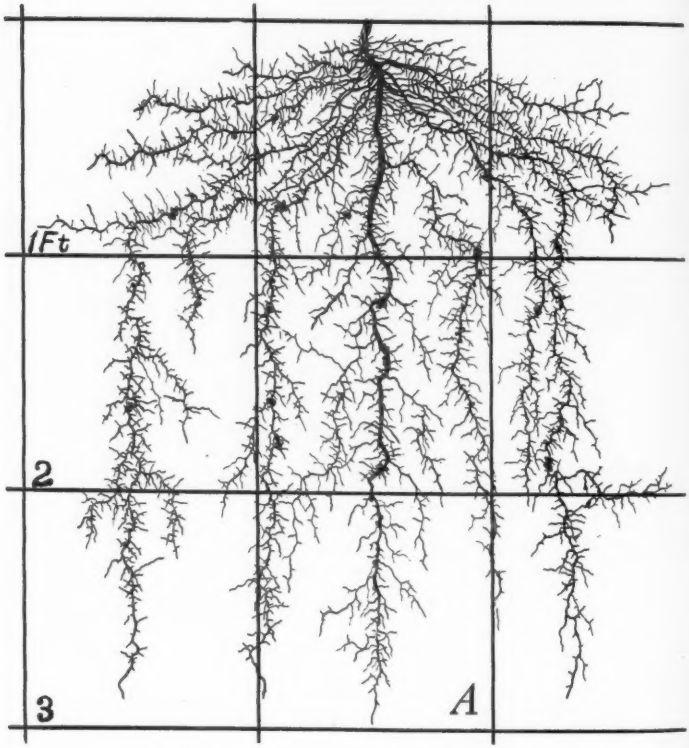

FIG. 2 *A*.—Tall pea root system: Telephone, parental

from 22 to 34 in., the average being 28.2 in. (table I and fig. 1 *A*). At 4–6 in. beneath the surface many large laterals took their origin from the tap root. Some of these ran off obliquely to a distance of 6–13 in., terminating at a depth of 5–8 in. below the surface. Others ran obliquely 6–9 in. from the vertical and then turned downward,

pursuing a course almost parallel with the tap and penetrating to a depth of 12–28 in. Many reached a depth of 24 in. The tap root as well as all of the main laterals was well supplied with branches

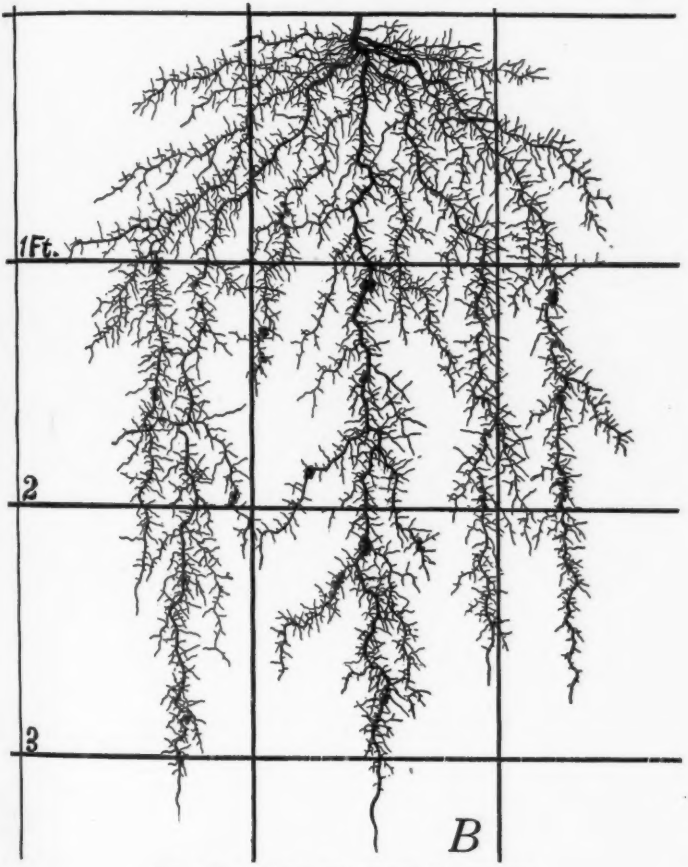

FIG. 2 *B*.—Tall pea root system: F_2 segregates

0.1–4 in. in length. There was an average of seven branches per linear inch. Some tertiary branches also occurred. The roots bore nodules rather sparsely to a depth of approximately 2 ft.

The parental Telephone peas, as compared with the Nott's Ex-

celsior, were slightly less mature when examined; however, some of the pods were white and crinkled, and little or no further growth occurred. An average of twenty plants gave a height of 46.5 in. (table I). The root system was similar in form to the smaller pea,

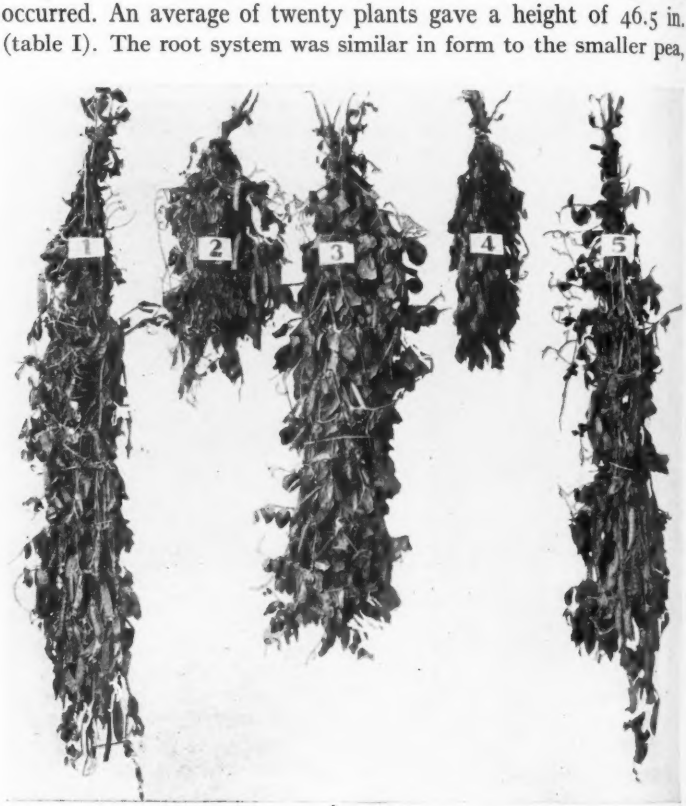

FIG. 3.—*1*, Parental Telephones; *2*, parental Nott's Excelsior; *3*, F_1 hybrid; *4*, dwarf F_2 segregates; *5*, tall F_2 segregates.

but all of its dimensions were greater (fig. 2 *A*). The maximum depth of penetration ranged from 28 to 45 in., with an average of 35.8 in. The smaller secondary branches were abundant, there being eleven per inch as compared with seven for the Nott's Excelsior. Nodules were also more abundant to a depth of over 2 ft.

The F_1 plants were in approximately the same stage of develop-

ment as were the parental Telephones. They were slightly shorter, but in other respects their above-ground characters were strikingly like those of the dominant parent. An average of twenty plants gave a height of 41.1 in. (table I). The root system was almost a counterpart of the larger pea in regard to form and branching. Its maximum penetration was a little greater, however, although its height of top was slightly less.

The F_2 generation of hybrids showed an almost exact Mendelian ratio in respect to height. Of the 277 plants that reached maturity, 209 were tall and 68 were dwarf (fig. 3). This gave a ratio of 3.07:1.

The dwarf F_2 segregates ranged in height from 14 to 26 in., with an average of 20 in. The tall F_2 segregates varied from 38 to 56 in., with a mean of 44.9 in. (table I). The smaller segregates had a root system almost identical with that of the recessive parent, except that its maximum penetration was slightly greater (fig. 1 *B*). These dwarf F_2's also bore nodules more profusely than did their similar parent, which may have been due either to some character inherited from the dominant line, or to their close proximity to the larger segregates, which, like their corresponding ancestor, produced tubercles to a marked degree. On the other hand, the tall segregate strongly resembled the dominant parent in respect to underground parts; indeed, the two root systems could scarcely be distinguished, except that the F_2 individuals penetrated the soil a little deeper. The maximum penetration of ten plants varied from 30 to 50 in., with an average of 40.9 in. (fig. 2 *B*).

Discussion

The data are limited to ten individual root systems in each case. The number was necessarily so restricted because of the time, labor, and expense involved. Since in the F_2 generation individual segregates were examined, it was frequently necessary to excavate a trench in order just to secure the measurements on a single root system. Conclusions drawn on the basis of this limited number of plants must be considered indicative and tentative only. Final conclusions must await further investigation.

An examination of the data suggests strongly the lack of any agreement between the ratios of root penetration to height of top

in the case of the two varieties of peas studied. For instance, the mean maximum root penetration and height for Nott's Excelsior was 28.2 and 19.2 in. respectively. This gives a ratio of 1.4. The Telephones, on the other hand, had a mean maximum root penetration of 35.8 in. and a mean height of 46.5 in. This gives a ratio of 0.7, which is only one-half as great as in the case of the small pea. The reasons for this lack of correlation may be due to several causes working either separately or in combination. It might result from a difference in the transpiring power of the tops, from a difference in the leaf areas, from a difference in the absorbing power of the roots, or from a difference in the branching habits of the roots which would affect the absorbing area. That this disparity between the root and top ratios is not a fortuitous one, however, is strongly supported by the F_2 segregates. Here the ratios are 1.5 and 0.9 respectively, which is almost the same relation as was found in the case of the parents.

Although the difference in root length between the two parental varieties is not great, the data show a strong tendency for such difference as there is to segregate along with height. In table I the range in height for Nott's Excelsior is found to be 14 in. with a mean of 19.2 in., and the variation in root length 12 in. with a mean of 28.2 in. The smaller F_2 segregates show a height range of 12 in. and a mean of 20 in., with a variation in root length of 12 in. and a mean of 30.9 in. Likewise, the parental Telephones show a height range of 18 in. with a mean of 46.5 in., and a root length variation of 17 in. with a mean of 35.8 in. The larger segregates have a height range of 18 in. with a mean of 44.9 in., and a root length variation of 20 in. with a mean of 40.9 in.

This close agreement in respect to the relation existing between height and root length, as between the parental lines and their F_2 segregates, might be due to a response to the transpirational demands of the plants, the taller tops requiring the longer roots. To decide this point the tops of ten parental Telephone plants were clipped off when they had reached a height of 9–10 in., and were kept pruned back to this height during the period in which the other plants were developing. The side shoots and flower buds that started on these pruned plants were pinched off as fast as they appeared, thus retaining as nearly as possible the original reduced area of

stem and leaves. Fig. 4 is a representation of the root system developed by these pruned plants. The measurements are given in table II. It may be seen by a comparison of fig. 4, fig. 2 *A*, and table II that, although the height of the clipped plants was only about

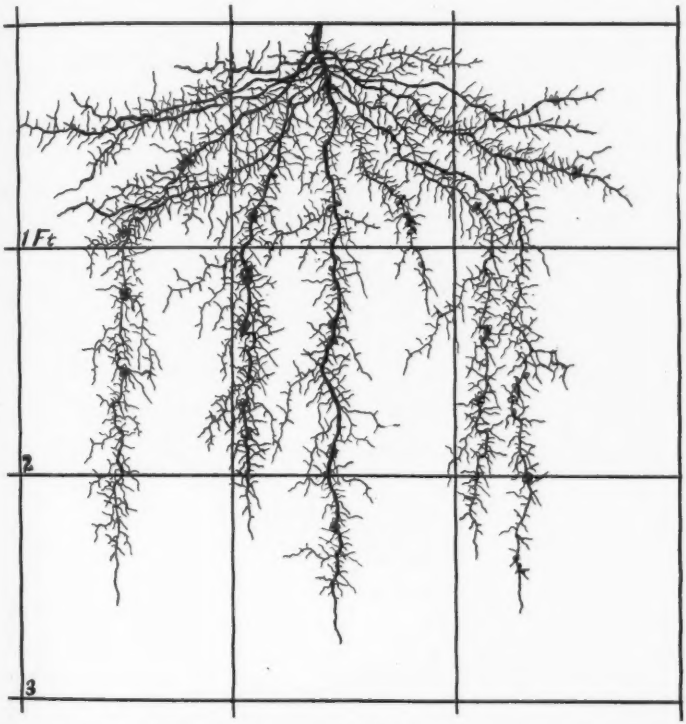

FIG. 4.—Root system of pruned Telephone plants; season 1926

one-fifth as great as those with normal tops, the root penetration was approximately within 2 in. as deep. Since these plants were legumes, taking part of their nitrogen supply from the air through their symbiotic bacteria, and also since the plot had been well fertilized, nitrogen hunger could have had little influence upon root growth (GERICKE **2**, REID **6**). The evidence, then, suggests strongly that hereditary genes accompanying those that determine height are the causative factors that determine root penetration in the

case of peas, and not the physiological demands alone of the above-ground parts.

The fact that pruned Telephone peas, in the summer of 1926, produced roots practically as long as those unpruned was unexpected, and it was decided to check this relation again in 1927. Accordingly, several hundred seeds were planted early in the spring, but these like a second planting failed to germinate. Twelve plants were finally secured. Eight of these were clipped at 11 in. and kept at this height; the other four were permitted to grow.

At the time of excavation the plants had reached approximately the same stage of maturity as those examined the previous year.

TABLE II

TELEPHONE PEAS

YEAR	PLANTS	NO. OF PLANTS	RANGE OF HEIGHT (IN.)	MEAN HEIGHT (IN.)	PERCENTAGE RELATION OF TOP HEIGHTS	RANGE OF MAXIMUM ROOT PENETRATION (IN.)	MEAN MAXIMUM ROOT PENETRATION (IN.)	PERCENTAGE RELATIONS OF ROOT PENETRATION (IN.)
1926.....	Unclipped	10	36 to 54	47.0	244.8	28 to 45	35.8	127.0
1926.....	Clipped	10	9 to 10	9.3	48.4	29 to 37	33.3	118.1
1927.....	Unclipped	4	36 to 41	38.8	202.1	34 to 49	43.3	153.5
1927.....	Clipped	8	0	11.0	57.3	34 to 47	39.5	140.1

Table II shows the top and root relations of both the clipped and the unclipped plants. The unpruned peas did not grow quite as tall as those of the previous year, but the roots of both the unclipped and the clipped ones penetrated more deeply. This was due to differences in the plots, which were a short distance from those of the previous year, the subsoil being less compact. The percentage relation of root penetration between the two lots, however, was very similar to that previously determined. This again indicates that root penetration in peas is determined by genetic factors rather than by the extent of the leaf area.

Had they been available, it would have been desirable in this investigation to use pure parental lines selected for uniformity of height. But, since peas are normally closely selfed, it is doubtful whether this condition would have changed the results appreciably. In so far as an examination of ten plants of each kind is significant, therefore, this work indicates that root length in peas, as well as height of tops, segregates in the F_2 generation; moreover, that these

top and root dimensions are linked in heredity. The data and figures also show that the root forms in the varieties studied are very similar, and that such differences as do exist are largely ones of magnitude.

Summary

1. There exists a great varietal difference in the ratio relation of root and top length between Nott's Excelsior and Telephone peas.

2. In the F_2 generation height of top and root length seem to segregate in almost the same relation that these characters existed in the parental stocks.

3. Failure to change the root penetration significantly in the tall parent by pruning the plants suggests genetic factors as the primary cause of root length.

4. In the two varieties studied the forms of the root systems are very similar, such difference as does exist being largely one of dimensions.

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CYTOLOGY OF HALIDRYS DIOICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 386

DOROTHEA G. DOUBT

(WITH SEVENTEEN FIGURES)

Introduction

The brown alga *Halidrys dioica* is a species of the Fucaceae which was described by GARDNER (5) in 1913, and is known only from the coast of southern California. Its range extends from Redondo to a short distance beyond San Diego, and includes Catalina Island. No study has been made of the species aside from the identification of a fungus and two brown algae that parasitize it (4, 17).

With the exception of the species *H. dioica*, *Halidrys* is confined to the coasts of northern Europe and the British Isles (6). DE TONI (20) reported its existence in the Japan and China seas, but the report has not been confirmed by recent workers (22). GARDNER distinguished *H. dioica* from the European species by four characters: smaller, wider-winged, more lanceolate, shorter stiped air vesicles; more profusely branched fruiting ramuli which frequently arise from the side of the air vesicles; more "zigzag" character of the stipe and lower branches; dioecious habit.

Described in 1819 by LYNGBYE (11) of Denmark, *H. siliquosa* was worked successively by GREVILLE (7), AGARDH (1), and THURET (19) during the first sixty years with comparatively small results, excepting the contribution of AGARDH to the knowledge of air vesicles; but the studies made since that time by OLTMANNS (13, 14), BOWER (2), REINKE (16), FARMER and WILLIAMS (5), LE TOUZE (10), and NIENBURG (12) have been fairly complete. OLTMANNS, who has assembled most of these data, notes that the growth of the plant is initiated by a 4-sided apical cell, that there is bilateral branching, that a midrib is gradually differentiated from the isodiametric cells, that a considerable development of hyphae takes place in the inner region, and that air vesicles develop about the midrib.

WILLE (21) and HICK (9) had worked on some Fucaceae and been successful in finding protoplasmic connections, but HANSTEEN (8) was not certain that the protoplasmic strands were carried through the pores he observed, and LE TOUZE thought there was no protoplasmic connection. LE TOUZE alone worked on *Halidrys*.

The products of assimilation have been studied repeatedly in *H. siliquosa* and in a number of other Fucaceae. CZAPEK (3) thinks the small, highly refractive globules which are very widespread in cells of the Fucaceae are tannin vacuoles, and are identical with the fucosan which HANSTEEN first thought was a carbohydrate and later a glucoside. CZAPEK refers to KYLIN'S work in which it was concluded that these bodies contain a phenol-like substance which is oxidizable, and which thus produces, after death, the coloring matter which has long since played so large a rôle in the chemistry of algae as phycophaein. CZAPEK credits MOLISCH and TSWETT with having discovered that it was a post-mortem product. Since CZAPEK'S work, PALMER (15) has shown that the material oxidized after death to produce the pigment phycophaein is a colorless chromogen in living cells, while the color of living tissue is due to a carotinoid, fucoxanthin. PALMER indicates that chlorophyll is also present in these cells.

BOWER (2), OLTMANNS (13), and NIENBURG (12) have worked out the development of the conceptacle in *H. siliquosa*. BOWER reported that an initial cell slightly sunken in the epidermis cuts off two or more segments by horizontal divisions. Then the basal cell divides, the first two walls being more or less vertical but inclined toward each other. It is some time before these cells divide in a plane parallel to the surface of the cavity. As a result, the conceptacle appears to be lined by a layer of cells continuous with the limiting layer, but as part at least of this tissue was derived from the basal cell, BOWER thought that this conclusion was not correct. Meanwhile the initial cell (or group of cells) has been completely thrown off by the swelling of the wall dividing it from the basal cell. Later, as in other Fucaceae, the cells of the lining tissue put forth papillae which develop further into hairs.

OLTMANNS' series runs very much like that of BOWER. An epidermal initial, more or less rectangular in shape, cuts off a basal

segment and becomes slightly sunken below the level of its neighbors. These lateral cells initiate the wall of the young conceptacle, but its growth is mainly due to divisions of the basal segment of the initial cells. The upper segment of the initial has meanwhile cut off several segments by horizontal division, forming a hair, as described by BOWER. OLTMANNS does not say that the hair is thrown off, however; he believes rather that when the conceptacle has reached its full size the hair is no longer distinguishable from the paraphyses; and while he is uncertain of the final disposition of the hair, it does not contribute anything significant to the development of the conceptacle.

NIENBURG made his report in 1913, and was influenced strongly in his study by the work of SIMONS on *Sargassum* (**18**). He found the conceptacle initial of *H. siliquosa* very similar to that of *Sargassum*, only more vacuolated, shorter, and the tongue narrower. As in *Sargassum*, he found that the first division of the tongue cell cuts off an inclosing lower cell around the base of the upper one; the second division cuts the basal cell in half longitudinally; the third cuts each of these cells in half obliquely toward the center of the initial; and the fourth divides the upper cell on each side of the initial. Further development is as outlined by OLTMANNS.

Materials and methods

The material used in this study came from San Diego, California. A number of plants were collected there in September, 1927, and fixed in a solution of formalin, acetic acid, and sea water.

A preliminary study was made of freehand sections, and later paraffin sections were used.

In preparing the material for slides, it was necessary to remove the air from the vesicles before imbedding. This was done when they were in the xylol, by piercing each chamber with a fine needle and exhausting the air from the inclosing flask by means of a force pump. Parawax melting at 52° C. was used as an imbedding medium, and sections were cut at 3 and 5 μ for most of the work; while a few topographical sections were cut at 10 μ. With the longitudinal sections, best results were gained when the material was cut parallel to the narrow surface.

Several stains were tested out, singly or in combination, but Haidenhain's haematoxylin and orange G were best for detailed work in this species. The quantities of granular and globular cell contents, which added not a little to the difficulty of effective staining, made little or no trouble when treated in this way. For topographical work the safranin and haematoxylin combination was good.

Investigation

The material used in the present study consisted of mature plants 75–85 cm. long. The male plants seemed to have considerably more abundant fruiting ramuli than the female, giving by comparison a very tufted appearance. It would be necessary, however, to examine a great many more specimens than were at hand to establish this character.

Air chambers were found developing in the leaves, in shoots that were elongated leaves, as separate air vesicles, and as portions of receptacle shoots (figs. 1, 2). Every possible intergrade was found between leaves and air vesicles. A leaf would give rise to another leaf or series of leaves, or to an air vesicle, or to both. There might be a single large chamber, or as many as three or more of them in the center of a very broad, flat leaf. Air vesicles of twelve chambers were frequently found, although eight or ten were most common. Groups of from one to fifteen chambers were noted in the portions of the shoots lying just under the receptacles. The shoots were uniformly green-brown in color, excepting the apices, which showed a lighter yellow tinge.

The assimilative and storage regions were examined, and found to contain mainly plastids of varying sizes and with colors from brown to green. The smaller plastids ranged down to the limit of vision, and were seen in stages of division. Especially large brown plastids averaging 7–8 μ in diameter were comparatively infrequent in the peripheral layer, but usually occurred in such quantities in the first two or three cells under it as to fill them to capacity, so that the nucleus was only occasionally visible. These plastids, called fucosan, have a very dense center, which is highly refractive when small and may appear cracked. They also have a more or less lamellated structure such as potato starch plastids have, although they do not

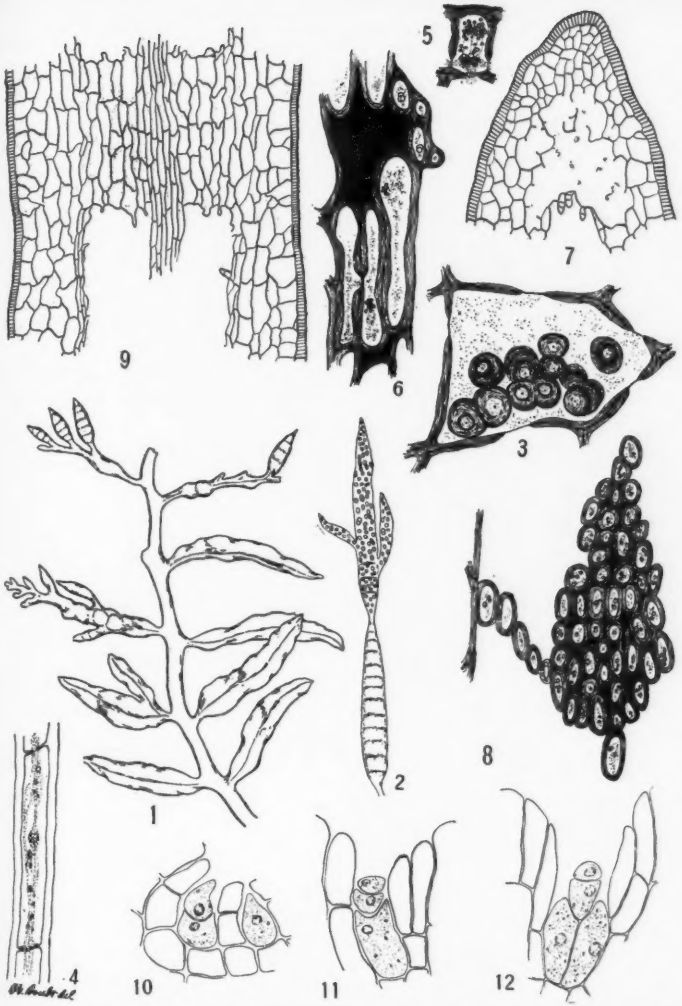
respond to a test for starch. They may be single, double, or compound (fig. 3). Occasionally the contents of the plastids are dissolved out, leaving only the honeycomb network of the plastid walls, which resists disintegration for a longer time.

Both lighter and darker plastids exist in large numbers in the peripheral cells, where they sometimes fill the entire space; but generally they can be seen to be connected by protoplasmic strands with the nucleus in older cells, or lying free in the vacuolated protoplasm of cells nearer the apex, in which they are much fewer in number. They are fairly numerous in the wall cells of the conceptacles but very rare in the pith. Only highly refractive, nearly colorless plastids are found as a rule in the paraphyses cells, although near the ostiole some shorter ones are quite packed with brown plastids. Hyphae seldom have these darker plastids, nor do the mature cells of the midrib. In these latter cells particularly, and elsewhere to a less extent, are to be found granules, globules, and crystals of many kinds, which react variously to stains and frequently do not stain at all.

The material was examined for protoplasmic connections, and these were found in every place in which they were sought. The cells of the midrib had sieve plates (fig. 4), and the paraphyses, peripheral cells, hyphae, and isodiametric cells had pores through which protoplasmic strands were seen to pass. The pores in the peripheral cells were at the base in lateral walls, one in each wall; there is but one pore in a wall in every case where pores exist, so far as known. The pore in the basal wall of a peripheral cell is generally in the center, but its position varies (fig. 5).

The walls are composed of many striated layers of material, as a rule divided by an extremely narrow middle lamella. Very frequently the angles of the cell walls have loosened and pulled apart, in which case the intervening space is filled with mucilage (figs. 6, 7). The walls of the inner isodiametric cells are particularly thick, and through them the middle lamella seems to connect across the pore, as if it alone composed the actual pore wall. Walls of every cell except in the paraphyses and apical region are quite thick.

The nucleus was found to be uniformly oval in shape, and to average 5 μ in diameter in every vegetative cell except those at the base of the apical groove. There they were larger, the apical nucleus



FIGS. 1–12.—Fig. 1, habit sketch showing intergrading between leaves and air vesicles, natural size; fig. 2, habit sketch of vesicular shoot terminated by receptacle, twice natural size; fig. 3, isodiametric cell containing fucoxanthin plastids; fig. 4, one of cells of midrib showing protoplasmic continuity through sieve plates; fig. 5, peripheral cell showing protoplasmic continuity through pores; fig. 6, lysigenous splitting of cells adjoining air chamber and entrance of hyphae elements; fig. 7, portion of cross-section of young air chamber showing breakdown of cell tissue; fig. 8, cross-section of midrib in chamber showing protruding filaments; fig. 9, longitudinal section of mature vesicular diaphragm showing condition of thallus cells and hyphae; fig. 10, single cell and 2-cell stages in development of conceptacle; fig. 11, 3-cell stage; fig. 12, 4-cell stage.[1]

[1] Drawings made with the Abbe camera lucida. Figs. 7, 9, 16, 17 magnified 150 times; figs. 6, 8, 650 times; figs. 3, 4, 5, 10, 11, 12, 13, 14, 15, 1250 times. The drawings have been reduced one-half.

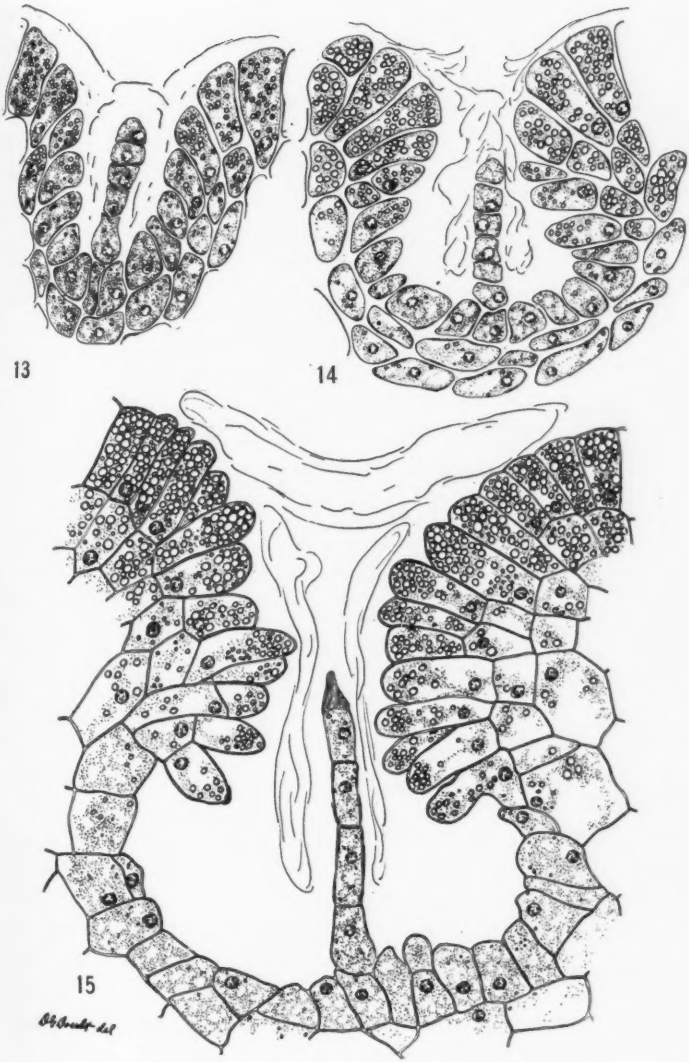
being nearly 10 μ in diameter. There was only one nucleus in every living cell of the plant so far as examined. This includes the cells of the midrib. The nucleus tends to occupy the center of the cell, except in the peripheral layer, where it seems always to be pressed down toward the base by the quantities of plastid material contained in the cell. In peripheral cells near the apex the nucleus still occupies a central position in the cell.

Hyphae arise as papillae from marginal cells of air chambers, or from cells of the midrib. In either case they are usually rounded in shape until the second cross wall has formed, after which they become filamentous. Young paraphyses frequently develop in the same way.

Hyphae are not numerous in *H. dioica*. None exist in the receptacles, except in old ones where air chambers are present or in process of formation. In fact, no hyphae have been found in this species except in the formation of or adjacent to air chambers. Hyphae are not found in the newer portion of the plant, but arise only after the midrib is well developed and the isodiametric cells are losing vitality. In addition it may be said that air vesicles do not develop in the newer portion of the plant.

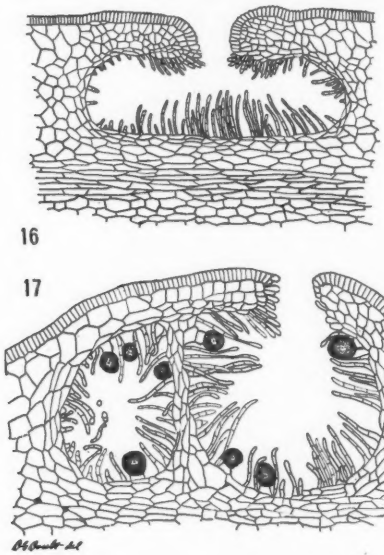
The conceptacle develops from a small initial cell, which becomes distinguishable about the third or fourth segment from the apical cell. Its base at that time is barely depressed below the neighboring cells, but the cell has taken on the characteristic tongue-shaped apex and is slightly shorter than the adjacent cells. Considerable mucilage is present in the apical groove, and separates the cells for several layers down into the thallus, so that no contact of adjacent cell walls is apparent near the base of the apical groove (fig. 10).

The conceptacle initial divides almost immediately, cutting off the tongue cell by a depressed **U**-shaped wall. Following that the basal cell merely elongates while the tongue cell divides twice horizontally (fig. 11). The adjacent cells are meanwhile growing and dividing, so that by the time the young conceptacle has been pressed upward to the top of the groove it is noticeably sunken in a small groove of its own, and the descendants of the tongue cell are free from the surrounding tissue. By this time a third wall has come in, separating the basal cell longitudinally into two equal parts, initiating the development of the wall of the conceptacle (fig. 12).



FIGS. 13–15.—Figs. 13, 14, further stages in development of hair and conceptacle wall; fig. 15, the hair and development of paraphyses in conceptacle.

Several successive divisions of basal and of tongue derivatives take place as the conceptacle recedes across the head of the shoot; and by the time it has reached the side of the receptacle the cells of tongue origin number five or six, and are depressed below the mouth of the young conceptacle by divisions of the basal cell. The cells at the base are becoming noticeably shorter and more numerous than



FIGS. 16, 17.—Fig. 16, later stage: paraphyses about half grown, and hair not distinguishable; fig. 17, oblique section of double, female conceptacle.

those near the mouth (figs. 13, 14). The basal cells have divided much more rapidly than their descendants and continue to broaden out the base.

As this trend of development continues, the mucilage which had been carried along in the conceptacle depression, between the walls of the adjacent cells, and particularly as a covering over the mouth of the depression, now becomes concentrated in small masses in the conceptacle and at its head, and it soon disappears. The tongue cell derivatives become elongated but give no evidence of further division, and the upper cell appears coated with mucilage. The rapidly

dividing basal cells begin to send out papillae and initiate paraphyses, while the more slowly growing cells of the neck, particularly the inner portion of them, loosen along their lateral walls and by successive divisions produce paraphyses also (fig. 15). After the paraphyses along the base of the conceptacle begin to grow there is no certain evidence of the tongue cell derivatives (fig. 16).

A peculiar condition has appeared frequently in this material. Two conceptacles develop fully, with their ostioles in different planes but with only three layers of cells separating them (fig. 17). Such a wall when seen in median section terminates abruptly at either end in a plane at right angles to the thallus. These double conceptacles are usually visible in habit material, as indicated in fig. 2.

Ectocarpus acuminata (17), previously described as a parasite growing in the conceptacles of *H. dioica*, was found quite frequently in the female conceptacles of this material. The sporangia were fully developed and beginning to shed their spores at the time of the extrusion of eggs from the conceptacle, and the vegetative cells were very few in number and scarcely distinguishable from cells of the rather abundant paraphyses. The spore nuclei were almost identical in size and appearance with those of the host plant.

Discussion

In appearance the material used was essentially as described by GARDNER (6), except for its greater size, more widespread and numerous air chambers, and differentiation between male and female plants. Growth was apical and followed the description of OLTMANNS (14) for *H. siliquosa*.

A study of the cell structure at once brought into evidence the plastid content, particularly the large dark plastids centering in the layer or two next under the peripheral layer. A comparison of the work of HANSTEEN, CZAPEK, and PALMER indicates that these plastids are what has been known as fucosan, and also that they contain fucoxanthin. Their size in excess of those in the peripheral layer may be accounted a result of food storage.

The existence of protoplasmic connections through the walls in various parts of the plant would agree with the findings of HICK in *Fucus* and *Ascophyllum*. Sieve plates had been found by WILLE in

some members of the Fucaceae, but LE TOUZE had disputed their existence in *Halidrys*. However, OLTMANNS and others considered that WILLE was correct. HANSTEEN also was not certain of protoplasmic connections through the pores of *Fucus*, *Pelvetia*, and *Sargassum*, but they were definitely distinguishable in the material of the present study of *H. dioica*.

The striated nature of the walls had not previously been noted. It was found to be a constant condition. It may be further remarked that the space left at the angles of the loosened cell walls had the appearance of mucilage rather than a much expanded middle lamella, as suggested by LE TOUZE. The idea proposed, that it is the middle lamella which connects across the pore opening, based on the results of staining, does not necessarily correlate with the statement of LE TOUZE that a portion of the original wall closes the pore, but depends on a determination of the origin of the middle lamella. The original wall may be involved in some way.

The lowered position of the nucleus toward the base in the peripheral cells is considerably more pronounced than the description of LE TOUZE for *H. siliquosa* would indicate. Also the existence of but one nucleus in the cells of the midrib is contrary to the report of OLTMANNS and WILLE for the Fucaceae in general, but coincides with the description of LE TOUZE for *H. siliquosa*. The larger size of the apical nucleus had not previously been noted. Otherwise the condition of the nucleus agreed with former descriptions.

The study of hyphae and air chambers showed, as has been found by previous workers, that they do not arise in the newer, more vigorous parts of the plant, but in regions which seem to be losing vitality. This loss of vitality may possibly be due to lack of food, or to pressure from some source or other, according to inferences made from this material. The fact that air vesicles do not arise in younger parts of the plant is attested, not only by the cell structure, but by the growth of deciduous receptacles, reported by GARDNER (6), above the air vesicles, and the fact that air vesicles never appear above receptacles. Moreover, the mucronate apices of air vesicles frequently contain normal apical cells, in which case early stages in the development of the conceptacle are to be found there, thus confirming the statement of AGARDH (1). But a majority of the vesicles

examined had been broken off, and either had the final chamber exposed or the organ had been grown over and the wound healed, as OLTMANNS described the recovery of wounded tissue in the Fucaceae (14).

Neither the exact cause of hyphae origin nor of the approximately periodic origin of air chambers has been investigated in previous studies. It is known that hyphae form a maze in the irregular mass of loosened disintegrated cell tissue. The cells of the midrib remaining in contact with the hyphae become looser, and while still rope-like, become divided into at least two strands. As observed in the present study, many horizontal hyphae filaments run out from these strands, connecting them with the receding hyphae, which now, like a wall, are proceeding where tissue breaks down but becoming attached where it holds (figs. 6–9). Since hyphae are conducting cells, according to WILLE, it may be supposed that they supply nourishment to the cells too distant from the outside or from light rays to acquire their own food, and thus hyphae maintain the cells in a stable condition, not subject to breakdown to any great extent.

It may be supposed with reason that though hyphae are conductors, the stimulus that causes them to start is chemical, and their first activity is resorbtion. If this be the case, another hyphae center will ultimately be formed a few cells above the now stable chamber, as a result of the breakdown of cell tissue at this spot. It is assumed that above a certain region the cells are not yet vulnerable to these new hyphae, and that on the other hand the basal diaphragm of the new chamber is already fairly well formed through the activities of the recent group of hyphae. The size of the new chamber then is predestined.

A study of the development of the conceptacle in this material brought out certain marked differences between some of its phases in this species and those which had previously been described for *H. siliquosa*. The initial cell agreed with the description of NIENBURG, in which he figures a tongue-shaped apex rather than the more or less rectangular cell of OLTMANNS (13) and BOWER. The first wall was curved, as NIENBURG has found, rather than according to the findings of BOWER or OLTMANNS, but there the resemblance to NIENBURG'S schedule ceased. The second wall, as has been noted, came

in horizontally in the tongue cell, rather than vertically in the basal cell. Even if it could be assumed that a division took place in the basal cell in the plane of the section, and thus not visible at this juncture, it would have been contrary to the sequence of NIENBURG, as would the division of the upper cell. It also has no parallel in the schedules of BOWER or OLTMANNS. Not only is the second wall located differently, but also the third comes in in a different sequence. The third wall in *H. dioica* comes in vertically across the center of the basal cell. This was the second wall in NIENBURG's series and in OLTMANNS', while it had no correspondence to anything in BOWER's series. According to BOWER, by comparison, there might be two or more straight, horizontal divisions of the initial cell before the vertical divisions of the basal cell began; then the first two walls of the basal cell were inclined toward each other.

Certain similarities as well as contrasts were observed between the behavior of the young conceptacle in *H. dioica* and in *H. siliquosa*, as described by the three previous reports. All are agreed that at least the base of the conceptacle is lined by cells derived from the basal portion of the initial, and that these cells produce paraphyses and gametes. Also all are agreed that the upper portion of the initial divides four or five times horizontally, producing a hair. BOWER does claim that this hair is early thrown off by the swelling of the wall dividing it from the basal cell, whereas it is otherwise agreed that the fate of the hair is uncertain; that it may remain in the mature conceptacle, indistinguishable from the surrounding paraphyses. Also, BOWER and OLTMANNS are not certain but that the peripheral cells adjoining the initial cut off some if not a considerable number of the upper wall cells of the conceptacle. There seems to be no evidence of that in *H. dioica*.

One phase of the development of the paraphyses had not been touched upon before, the fact that the upper wall cells split laterally and grow out as paraphyses rather than forming papillae as an initial step. This splitting may be accounted for by the fact that the growth tendency is toward the ostiole, and does not press them as firmly as the cells are pressed on the base of the chamber (fig. 15).

Finally, the development of double conceptacles had not previously been noted. It seems likely that the situation in fig. 2 fre-

quently occurs, in which two successive initials are separated by only one wall. It seems reasonable to suppose that the pressure of growth exerted by these two young initials and their adjacent cells inhibited division in the vertical, though not in the horizontal nor longitudinal planes in the basal cell of this single wall layer; and that later, as the number of segments from the basal cell of the initial multiplied, they spread over the surface formed by this wall, which grew at a corresponding rate, and the outcome was a 3- or 4-layered diaphragm between the two conceptacles.

Summary

1. There is an unbroken series of intergrades between a leaf and an air vesicle in *Halidrys dioica*.

2. The origin of air chambers seems to be a matter of food relationships in which hyphae play an important part.

3. The fucosan of HANSTEEN seems to be a fucoxanthin plastid.

4. Protoplasmic connections are continuous throughout the plant.

5. The early development of the conceptacle stresses the upper rather than the basal cell, as preceding work on the European species had shown.

6. The frequent appearance of double conceptacles may be due to the action of two initials upon a single intervening wall cell.

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EFFECT OF SULPHATE ON LEMON LEAVES[1]

A. R. C. Haas and E. E. Thomas

(with two figures)

It is well known that the irrigation waters of citrus areas contain more or less salts; even those used on the very best citrus groves contain ordinarily some chloride and sulphate (6). These in small to moderate amounts are not particularly objectionable, but large amounts of chloride or sulphate not only bring about the accumulation of toxic concentrations within the leaves, but also reduce the absorption of nitrate by the plant (3).

Analyses of lemon leaves from soils irrigated with water containing large amounts of calcium sulphate have shown that severe injury by accumulation of sulphur compounds in the leaves is accompanied by a reduced amount of total phosphorus, represented almost entirely by the decrease in inorganic phosphorus. Conversely, the toxicity can be somewhat decreased by generous additions of nitrogen fertilizers. These bring about a greater leaf area with a consequent reduction of the concentration and toxicity of chloride or sulphate in the leaves.

The occurrence of large amounts of chloride or sulphate in the irrigation water is accompanied by high concentrations of sodium, calcium, or magnesium. The effects of the accompanying bases are the more favorable when the bases are divalent rather than monovalent. Often the choice of fertilizer materials is such that considerable amounts of chloride or sulphate are applied either unintentionally or without knowledge of the danger of injury that may ultimately result.

Experiments have been conducted with the use of sand cultures in order to observe the effects of sulphate upon the growth of citrus. Twelve ten-gallon crocks with a hole in the bottom for drainage were employed as the culture vessels. Crushed quartz was placed in the bottom of each crock, and pure silica sand containing one

[1] Paper no. 186, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

pound of pure calcium sulphate was placed in each crock. Budded lemon trees washed free of adhering soil were planted in the sand after being pruned free of all foliage and most of the smaller roots. The trees were allowed to grow in these containers while receiving a culture solution identical with that given to series I in table I. Iron in the form of ferric tartrate, and a solution of "A-Z" (2) were added to the culture solutions, so as to give a concentration of 0.2 ppm of certain constituents.

Instead of producing normal growth as in controls that received Hoagland's culture solution, many of the trees lost a considerable

TABLE I

SAND CULTURES: CULTURE SOLUTION ADDED TO SAND CONTAINING CALCIUM SULPHATE

SERIES	PARTS PER MILLION IN CULTURE SOLUTION ADDED						
	Na	K	Ca	Mg	NO_3	SO_4	PO_4
I	61	22	.....	57	164	223	35
II	61	22	477	57	1643	223	35
III	61	22	795	57	2629	223	35

number of their leaves at the end of the first year of growth, so that the shoots were bare of leaves except near the tips. In some cases the entire shoots were defoliated, and some of the leaves turned greenish yellow, while others mottled somewhat and became yellow or golden colored at the tip prior to abscission. A composite sample of mature leaves from all twelve of these trees was taken for analysis.

The twelve containers were then arranged in three series, which received the culture solutions described in table I. The calcium sulphate served as the only source of calcium in the first series of four crocks, while in the other two series of four crocks each the culture solutions contained additional calcium as calcium nitrate. The pH of the solutions added was close to 7, and that of the drainage water ranged from pH 4.8 to pH 6.6. Acidity of the culture solution as a result of absorption or excretion by the roots and the possibility of its toxic action upon growth are therefore factors involved in studies pertaining to calcium sulphate. We have eliminated much

of this acid toxicity by the frequent use of solutions of high pH values.

In series II and III the leaves were retained on the shoots for much longer periods than in series I, although gradually much of the distal portion of the shoots became bare of leaves. In series II and III leaves of a certain type were affected by mottling with a yellowish or golden colored leaf tip (fig. 1). As the leaves became mature, the yellowish mottling tended to become somewhat bronzed. Also brown spots dotted the margin and the mottled area on the ventral side of the leaves. In some cases actual burning or drying out of the margin of the leaves occurred. These effects of sulphate resemble those caused by an excess of boron. The latter, briefly alluded to in a preliminary announcement by KELLEY and BROWN (4), will be more fully discussed by them in the near future.

Analysis of a composite sample of mature leaves from the trees, before dividing the containers into three series, showed that the dry matter of the leaves contained 1.57 per cent of sulphur, which is equivalent to 4.70 per cent of SO_4. The ash of the water-soluble fraction was 8.92 per cent, and that of the water-insoluble fraction was 10.10 per cent of the dry matter. The ash of the water-soluble fraction was 46.89 per cent of the total ash of the leaves, which is quite abnormally high for mature lemon leaves. HAAS (1) has shown the ash of the water-soluble fraction of normal, mature lemon leaves from the plots on the Rubidoux tract of the Citrus Experiment Station to be about 30 per cent of the total ash.

The total calcium of the lemon leaves of the trees in the original calcium sulphate series was 27.22 per cent of the total ash, which is typical of mottled leaves (5). The calcium of the water-soluble fraction of normal lemon leaves has been found by HAAS (1) to be about 17–18 per cent of the total calcium, whereas 30.93 per cent of the total calcium of these lemon leaves was water soluble. The magnesium, sodium, and potassium percentages were about the same as for normal lemon leaves. It is quite possible that the high content of sulphur in the leaves may have brought about an increased solubility of the calcium because of increased acidity of the tissue, although such sap studies have not been made as yet.

In the sand cultures just described it was observed that, with

increasing concentrations of calcium nitrate in the culture solution, the lemon leaves presented a darker green color than where no addi-

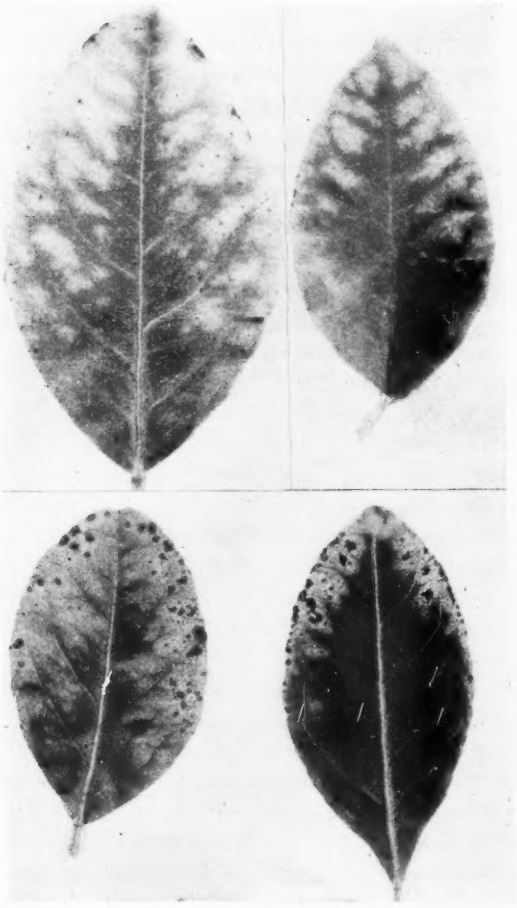

FIG. 1.—Effects produced upon lemon leaves from trees in sand cultures containing large concentrations of sulphur: upper row, dorsal surface; lower row, ventral surface.

tions of calcium nitrate were made. The trees to which calcium nitrate was added made fair growth and retained their leaves, while

those that did not receive the calcium nitrate made practically no growth and lost nearly all of their leaves. There is reason to believe that the absorption in increasing amounts of any other anion will reduce the toxic effect of the absorbed SO_4. This was quite evident from the effect on lemon trees in several series of sand cultures in galvanized iron containers 20 inches in diameter and 26 inches deep. In one case 500 gm. of calcium sulphate was mixed with the sand in each container, and in another case 500 gm. of calcium sulphate plus 500 gm. tricalcium phosphate (table II).

TABLE II

SAND CULTURES WITH NUTRIENT SOLUTION AND ADDED SALTS

ADDITIONS TO SAND	PARTS PER MILLION IN CULTURE SOLUTION ADDED TO SAND							
	Na	K	Ca	Mg	Cl	NO_3	SO_4	PO_4
$CaSO_4$	7	184	.....	54	10	225	216	105
$CaSO_4+Ca_3(PO_4)_2$	7	142	.....	54	10	225	216	.....

Each series of sand cultures included budded lemon trees, and received all of the elements necessary for growth. In the calcium sulphate series the leaves were a bronzed greenish yellow, with many of the shoots bare of leaves. Where tricalcium phosphate and calcium sulphate were added to the sand the leaves were somewhat mottled, as were those in the preceding experiment (fig. 1). The trees grown in the first series made practically no growth and lost nearly all of their leaves, while those in the second series made very good growth and retained their leaves.

It was observed that in many of the fertilizer plots of the Rubidoux tract of the Citrus Experiment Station, the lemon shoots had lost many of their oldest leaves; and many of the leaves still attached were almost indistinguishable from the leaves of trees which received a large amount of sulphur in sand cultures. Moreover lemon trees on unfertilized plots showed no such effects. At once it was surmised that possibly accumulations of sulphur had occurred in the leaves.

The concentration of SO_4 in the soil solution in the various plots is subject to considerable change throughout the year by rainfall,

irrigation, and cultural operations, as well as by root absorption from the soil solution. It is of interest that the lemon leaves from trees of plots D, E, J, K, N, and R, which received applications of fertilizers containing sulphate,[2] showed visible evidence of injurious effects, while lemon leaves from trees on the unfertilized plots B and M showed no obvious symptoms. It is of added interest that lemon leaves from trees on plots A, L, and Q, which received as much if not more SO_4 than some of the other plots, showed no symptoms of sulphate accumulation.

Analyses were made of mature lemon leaves collected in August, 1927, from the various fertilizer plots of the Rubidoux tract. Total chlorine was determined by the sodium carbonate method, and total sulphur by the magnesium nitrate method. The results are given in table III, each percentage representing the average of two or more closely agreeing duplicate determinations.

It will be noted that the chlorine concentration is nowhere excessive, and that the dry matter of lemon leaves from trees of plots A, L, and Q contains approximately the same amount of sulphur as that from plots B and M. The concentrations of sulphur found in lemon leaves from trees in plots A, L, Q, B, and M agree very well with the results obtained by KELLEY and CUMMINS (5) for normal mature lemon leaves. The dry matter of the lemon leaves from trees of plots D, E, J, K, N, and R, however, contains more sulphur in each case. Fig. 2 shows lemon leaves in which the accumulation of sulphur was noted.

In examining table III the question may be raised as to why the lemon leaves of trees on plots A, L, and Q show such a low concentration of sulphur and consequent lack of injury, in view of the fact that considerable sulphur is added in their fertilizer treatment. This but emphasizes an important consideration mentioned at the outset, namely, that additions of fertilizers that may introduce large amounts of SO_4 ions in the soil solution may bring about considerable injury (plots D, E, J, K, N, and R). If such fertilizers be supplemented by fertilizers that add large amounts of ions other than sulphur to the soil solution, these latter tend to annul some or all

[2] A water extract of "fine steamed bone meal" used on plot E showed copious amounts of sulphate.

of the bad effects that otherwise would have been produced. The addition, therefore, of certain fertilizers that supply nitrate, etc., to soils rich in sulphur may have their beneficial effect partly by causing the trees to produce more growth, with a consequent reduction of the sulphur in the leaves to the point where it has no toxic effect. Growth of new leaves, therefore, acts to dilute and reduce the toxicity of sulphur within the leaves.

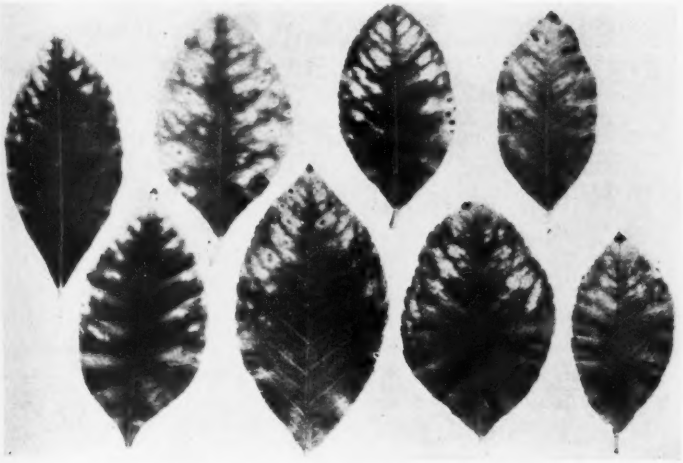

FIG. 2.—Discoloration of lemon leaves that accompanies sulphur accumulation; leaves taken from Rubidoux Tract, Citrus Experiment Station.

This discussion may bring a partial answer to the oft repeated question as to what concentration of nitrate shall be maintained in the soil solution for the best condition of citrus. There seems to be no one definite concentration of nitrate in a soil solution that can be said to be best for citrus on any soil type, or on the same soil type under different types of fertilization. The concentration of nitrate or any other constituent that is most desirable in the soil solution is dependent, in part at least, on the concentration of the other constituents of the soil solution and upon their ease of displacement and rate of renewal.

In certain lemon-producing districts it has long been known that

TABLE III

TOTAL Cl AND S CONTENT AS PERCENTAGE DRY MATTER OF LEAVES OF LISBON LEMON TREES FROM PLOTS OF RUBIDOUX FERTILIZER TRACT (EUREKA LEMON ADDED FOR ONE PLOT)

PLOT	A	L	Q	B	M	D	E	J		K	N	R
Fertilizer treatment	Complete: nitrate of soda, blood, steamed bone meal and sulphate of potash	Nitrate of soda, blood, and sulphate of potash	Complete: nitrate of soda, blood, superphosphate and sulphate of potash	No fertilizer	No fertilizer	Sulphate of potash	Steamed bone meal	Superphosphate		Steamed bone meal and sulphate of potash	Superphosphate and blood to equal nitrogen in bone plots	Sulphate of potash
								Eureka	Lisbon			
Total Cl.....	0.09	0.10	0.10	0.06	0.05	0.04	.....	0.05	0.04	0.03	0.04	0.05
Total S.....	0.40	0.32	0.44	0.46	0.50	0.80	0.82	0.88	1.10	0.88	0.93	0.68
Symptoms of S accumulation in leaves.....	Absent	Absent	Absent	Absent	Absent	Severe	Severe	Severe	Severe	Severe	Severe	Severe

the irrigation water is comparatively rich in sulphur, largely in the form of calcium sulphate. Frequently leaves of such lemon trees are tip-burned, and have a type of mottling resembling that in fig. 1. Leaves from one location showed the total sulphur calculated as SO_4 to be 3.14 per cent of the dry matter, while ordinarily the normal leaves of good groves contain about 0.96 per cent. The inorganic SO_4 constituted 12.89 per cent of the ash, or 2.52 per cent of the dry matter; while the Ca was 32.6 per cent of the ash, or 6.38 per cent of the dry matter. Leaves from another location contained 9.09 per cent of SO_4 and 23.78 per cent of Ca in the ash. The total sulphur calculated as SO_4 was 1.80 per cent of the dry matter, while the Ca was 3.30 per cent. The leaves were of full size but severely mottled and tip-burned, the mottling being accompanied by a bronzed coloration. These results indicate that accumulations of sulphur may occur in lemon leaves when the trees are irrigated with water containing appreciable amounts of sulphate.

Summary

1. In controlled sand cultures it was found that large concentrations of sulphate in the solution bathing lemon tree roots may bring about toxic effects in the leaves.

2. These effects are characterized by a type of mottling with a yellow or bronzed coloration and sometimes burning of these areas. This is accompanied by marked abscission of the leaves.

3. In sand cultures a reduction in toxicity due to sulphate was brought about by increased concentrations of phosphate and nitrate.

4. Field investigations upon lemon leaves have confirmed observations previously made with sand cultures, and have shown the ameliorating effect of supplementary fertilization that brings about increased tree growth with a reduction of the concentration of accumulations within the leaves to a point where toxicity is not evident.

5. Presumably there is no one general optimum concentration of nitrate for the growth of citrus. The desirable concentration depends upon many factors, such as the concentration of other constituents of the soil solution and their ease of renewal.

6. Irrigation supplies that are rich in sulphate may cause more or less injury to lemon trees, especially where the available nitrogen is insufficient.

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INJECTION METHOD AS A MEANS OF IMPROVING CHLOROTIC ORANGE TREES[1]

E. E. Thomas and A. R. C. Haas

(with three figures)

Chlorosis of citrus trees, although widespread throughout the citrus-growing areas of California, is not of a very serious nature except in restricted localities. Its occurrence may be associated with various soil conditions, but frequently the complications are such that it is difficult to determine the causal factor. The chlorosis of citrus often occurs on soils that are rich in calcium carbonate or other carbonates. Such soils usually have a high enough hydroxyl-ion concentration to precipitate out in the soil or within the plant the iron salts so essential for the production of chlorophyll. Frequently such soils are rich in the constituents necessary for growth, but some of them are made unavailable for the normal growth of the plant. In such areas the drainage conditions may be of the very best, and yet chlorotic growth appears as a consequence of the alkalinity due to the calcium carbonate.

Our first effort to remedy chlorosis in citrus plantings was confined to lemon trees, where large applications of iron sulphate were applied directly to the soil. The applications varied in amounts from 6 to 25 pounds per tree, and the material was applied in various ways, namely, in holes, in furrows, and in basins where the surface soil was temporarily removed. An adequate number of chlorotic lemon trees were selected as controls, and kept under observation during the time of the experiment. Observations were made at intervals over a period of five years, and the conclusion was reached that no definite improvement of the trees could be attributed to the applications that were made.

Further observations have been made on orange trees growing on soil which is rich in calcium carbonate. Here the applications of iron sulphate have been made in amounts varying from $12\frac{1}{2}$ to

[1] Paper no. 184, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

100 pounds per tree. The material was broadcast and thoroughly incorporated with the surface soil. No obvious improvement of the trees as a consequence of the treatment could be noted.

The writers have recently limited their experiments on chlorosis almost entirely to the injection method as a means of treating Valencia orange trees on soils known to have a high calcium carbonate content.

The method employed is in many respects similar to that previously employed by GORDON and LIPMAN (1) and LIPMAN and GORDON (3, 4), but with certain modifications. In their method a single hole was bored into the trunk of the tree, almost at right angles to the direction of the trunk, and a glass tube was sealed into the hole with wax. This glass tube was attached by means of a long rubber tube to the reservoir containing the solution.

In our method we have substituted a galvanized iron pipe for the glass tube. This iron pipe is threaded at one end, screwed into the hole for a short distance and sealed by means of beeswax.

In our initial experiments with this method we employed but one hole for the injection of the solution into the tree, and iron tartrate as well as iron sulphate was used. Whenever chlorotic trees were injected, adjacent chlorotic trees were kept under observation as controls. This is a very important consideration when trees are observed over a period of years, in order that any improvement that may result from causes other than those brought about by the injection may be evident.

Injection experiments have been made on Valencia orange trees at different seasons of the year, and it has been found that the greatest response to the treatment occurs when it is given just prior to the beginning of new growth in the spring.

Previous investigators (MILAD 6, and MARSH and SHIVE 5) have found no deficiency of iron in chlorotic plants, but rather a non-uniform distribution of that which is present. In order to determine whether the iron present in chlorotic citrus trees can be made soluble and be utilized by the chlorotic foliage, therefore, we have injected into branches of chlorotic orange trees solutions of citric and of tartaric acid, in concentrations ranging from 1 to 9 gm. in two liters of distilled water, with no positive results.

Valencia orange trees about 20 years of age were each injected by the single-hole method with two liters of distilled water containing 10 gm. of iron tartrate. As a result of this single injection there was a marked abscission of the older chlorotic leaves, followed by a production of new growth from the axillary buds. The new growth was of normal green color and a considerable increase in the fruit production was observed. This immediate improvement in the condition of the trees confirms the results of LIPMAN and GORDON (3). Frequently, however, a single branch in a tree was conspicuous in that it showed no effect of the treatment. In less than two years the leaves of the succeeding cycles of growth were of a pale color, indicating the transient nature of the effect.

By the single-hole method we have also injected Valencia orange trees in the same grove with 3, 5, or 7 gm. of iron sulphate in two liters of distilled water. In these experiments, in contrast to those in which iron tartrate was used, the foliage of those branches only that were above the hole showed the effects of the treatment.

In the case of iron sulphate, there was not as marked an abscission of the older chlorotic leaves as in the case of iron tartrate, and the apparent improvement of the tree appeared to be more gradual, since the old chlorotic leaves were retained for a much longer time.

As a result of these observations it was evident that, in order to affect the entire tree by these iron salts, it would be necessary to inject the solution either in or below each main branch. Experiments were therefore begun in which a hole was bored below each main branch. Two liters of solution containing 1–2 gm. of iron tartrate was injected below each main branch. The trees employed had from three to five main branches. Subsequent observations showed that such treatment was very effective. There was marked abscission of the older chlorotic leaves, and the new growth produced had the green color of normal leaves. The subsequent cycles of growth, however, became increasingly chlorotic.

A similar experiment with iron sulphate was conducted in the same grove. Two liters of solution containing 1.5–2 gm. of iron sulphate was injected below each main branch. The trees selected had from three to seven main branches. As already stated, the effect on the trees was much less marked than with similar concentrations

of iron tartrate, but the trees showed considerable improvement in color as the old chlorotic leaves gradually abscissed. The improvement of the trees was of comparatively short duration, in that succeeding cycles of growth became increasingly chlorotic.

Since the effect of the treatment was found to be temporary it became necessary to reinject the trees. It was found impossible to reinject them with solution by making use of the holes previously employed. This was true whether free circulation of air was permitted into the holes or whether the holes were sealed after the first injection and then reopened for the second treatment. The failure of the tree to absorb solution from the second application in the same hole bore no relationship to the concentration of the solution employed. It has been impossible to obtain absorption in the same hole from successive injections of water or of weak concentrations of iron solutions. However, if the hole is enlarged, some further absorption of solution takes place, but this may be extremely small in amount and insufficient to prevent the foliage from again becoming chlorotic. If new holes are bored on the same horizontal plane as that of the original holes, more solution can be absorbed by the tree with beneficial results. After making several applications in this way, it becomes necessary to bore new holes in the same longitudinal plane as that of some previously bored. In this case we have found that it is difficult if not almost impossible to secure absorption from these new holes. This is true whether the holes are placed above or below the original one.

An investigation as to the cause of the lack of absorption from new holes made in the same longitudinal plane as those previously used was then conducted. On boring the new holes it was noticed that the wood was of a darker color than that of untreated trees. Trees injected in this way were sawed off at the ground level and the trunk and lower portions of the main branches were then cut in various planes. Fig. 1 illustrates two transverse sections of the trunk of an injected tree. It shows the callusing-over of a hole shallower than that ordinarily employed. Even though the exterior of the hole was immediately sealed after the injection was completed, the inside of the hole is almost completely filled with fungus. The hole is bounded by a narrow margin of dead and discolored tissue.

The figure shows the location of four shallow holes, and shows that there is very little lateral movement of the injected solution; this condition explains the fact that only those branches above the hole are affected by the injection.

A longitudinal section of the trunk of a Valencia orange tree that has been twice injected in new holes in the same vertical plane is illustrated in fig. 2. The discoloration of the tissue brought about by the injection of the iron-salt solutions is very conspicuous. The

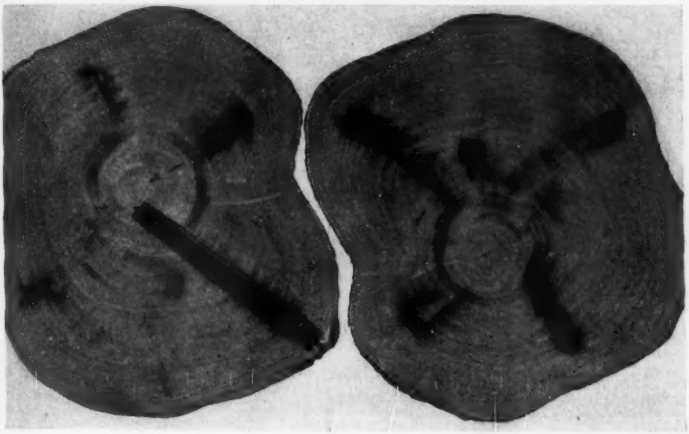

FIG. 1.—Transverse sections of trunk of injected orange tree, showing callusing over of hole and discoloration of wood in longitudinal plane brought about by injection of iron-salt solutions.

plane parallel to the long axis of the holes shows the marked upward and downward movement of the solution. This same plane shown in the photograph is perpendicular to other holes, and shows the small lateral movement of the injected solution.

The upward and downward movement of the injected iron solutions (fig. 2) is so pronounced that its effects frequently are visible in the main branches of the tree, as is well illustrated in fig. 3. A line connecting the darker areas in this transverse section lies in the same longitudinal plane as the hole in the trunk of the tree.

When concentrations of iron salts such as we have employed are used, no symptoms of internal injury are manifest on the ex-

terior of the trunk. With higher concentrations, however, a strip of bark may be killed for considerable distances up the tree. This killing of the bark is accompanied by an exudation of gum.

We have found in these studies that a concentration of 1.5 gm. of iron sulphate in two liters of distilled water has proved beneficial to chlorotic trees, while much smaller concentrations of iron sulphate have not proved effective in severe cases of chlorosis. This concentration, however, has produced the injurious effects described.

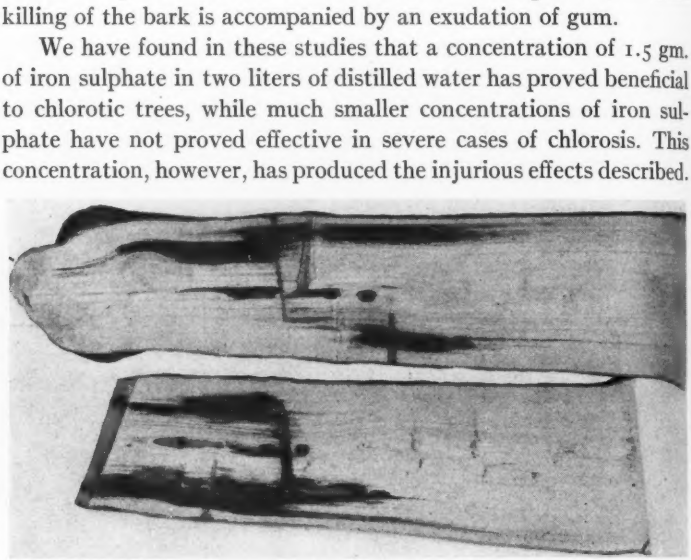

FIG. 2.—Longitudinal sections of orange tree trunk injected with iron-salt solution; discoloration shows the upward, downward, and slight lateral movement of iron-salt solutions.

It is of interest, therefore, to ascertain what effect an injection of 1.5 gm. of iron sulphate would have upon the tree when the salt was dissolved in 36 rather than 2 liters of distilled water. This experiment has shown that an orange tree 20 years of age could absorb but 18 liters of such a solution. Efforts to secure further absorption in the same hole, or in holes above or below the original hole but in the same vertical plane, have proved futile. An examination of the wood has shown a discoloration characteristic of iron salts.

This discoloration of the wood is in decided contrast with the normal color which persists after an injection of salt solutions other than those of iron. This was found to be the case in studies on the effect produced by the injection of large volumes (18 liters or more) of double-strength Hoagland's solution, and also by the injection of large volumes (6 liters or more) of calcium nitrate solution (800

parts per million of calcium) into badly mottled orange trees. No effects of such treatments were apparent.

The results we have obtained by the use of the injection method in the treatment of chlorotic orange trees confirm those reported by LIPMAN and GORDON (3) for lemon trees, so far as the initial effect upon the trees is concerned. Their conclusions, however, in regard to the injection method as a cure for chlorosis of lemon trees have not been confirmed by our results with orange trees.

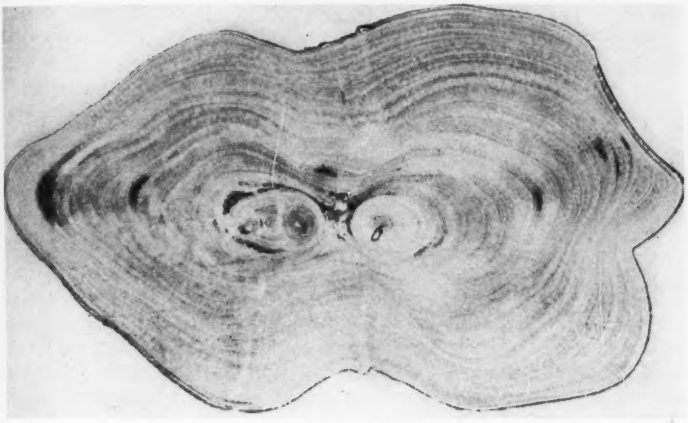

FIG. 3.—Transverse section of main branches of injected orange tree immediately above the trunk, showing pronounced upward movement of injected iron-salt solution.

In 1921 HENDRICKSON (2) injected pear trees growing on calcareous soil with iron sulphate solution through an inserted tube. After using various concentrations, he decided that without further work tree injection could not be recommended as a practical control for chlorosis.

In view of the temporary nature of the benefits to be derived from injection of orange trees with iron-salt solutions, the killing of certain tissues of the trunk with the possible accompaniment of fungous infection, the weakening of the tree, and the expense of such treatment, further investigation is necessary before tree injections with iron salts may be recommended as a cure for chlorosis of orange trees.

Summary

1. Chlorotic orange trees have been improved temporarily by the injection of iron-salt solutions into the trees. Subsequent injections are necessary in order to maintain the trees in a healthy condition. The difficulty, however, lies in the fact that the same hole cannot be used for the subsequent injections. This is due to the fact that iron salts in the concentration required to improve the tree discolor and kill the wood for a considerable distance up and down the trunk.

2. In addition to the weakening of the tree, it is very likely that fungi will develop in the injection holes.

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CURRENT LITERATURE

BOOK REVIEWS

Gasteromycetes of eastern North America

A comprehensive, modernized treatment of American Gasteromycetes has long been needed. MORGAN'S work is incomplete, it is not readily available to many workers, and his nomenclature is obsolete. The same criticisms apply to the writings of LLOYD and of PECK. The beautifully illustrated and very complete account of the Gasteromycetes of the eastern United States and Canada by COKER and COUCH,[1] therefore, will be eagerly welcomed by students of the fungi everywhere, but especially by those residing in the region covered or working on its fungus flora. While this region is not clearly delimited, it apparently includes continental North America east of the great plains. Over 150 species are recognized as occurring within this territory, and are described fully. There are, in addition, numerous critical notes on extra-limital species which add greatly to the value of the work.

The taxonomic treatment is conservative. One new genus, *Nigropogon*, belonging to the Hymenogastraceae, and 11 new species and varieties, mostly included in the same little known family, are described. This is surely not excessive in a work of the scope of the one under consideration. The Arachniaceae is apparently here proposed as a family for the first time, although the fact is not indicated. The authors suggest that *Astraeus* might well justify another family; but in this case, as in the instance of several doubtful species where new names could easily have been defended, they exercise commendable restraint. Had other authors been equally considerate, the problem of dealing with synonyms would be simpler.

The plates include reproductions of photographs, photomicrographs, and drawings, and illustrate both habit and microscopical details of the great majority of the species discussed. A few of the photomicrographs add little to the value of the book, but with this qualification, the illustrations represent a notable contribution to the morphology as well as to the taxonomy of the group.

The authors nowhere suggest the taxonomic rank they would assign to the Gasteromycetes as a whole; they recognize no subdivision larger than a family, and the arrangement of the families sheds little light upon their views concerning inter-relationships within the group. While comments bearing upon this problem occur here and there throughout the course of the systematic treat-

[1] COKER, WM. C., and COUCH, J. N., The Gasteromycetes of the eastern United States and Canada. 4vo. pp. ix+195. *pls. 123*. Chapel Hill, University of North Carolina Press. 1928.

ment, it is to be regretted that they have not seen fit to summarize their conclusions. The reviewer is inclined to characterize this lack as the one really disappointing feature of the book. A few details are subject to criticism. For example, the habitual reference to the basidiocarp as the "plant" has a certain amount of usage to justify it, but little else. The statement that MORGAN'S specimens have been destroyed is fortunately incorrect. These have nearly all been preserved, and are now in the possession of the State University of Iowa, where, it is expected, they will soon be available for reference. The statement concerning the liberation of the spores of the Nidulariaceae (p. 131) is pure assumption. There is no evidence that in the natural course of events the spores of these fungi are ever liberated from the peridioles. In a few cases the keys do not seem to be clear. These are minor points, however, and it would be unfair to stress them in discussing a work which includes such a wealth of new and critical information concerning nearly every species discussed as to make it one of the outstanding contributions to mycological literature of recent years.

The bibliography seems to be complete, and, as in the case of the earlier works on the water molds and the Clavarias from the same source, the volume is beautifully printed, and remarkably free from typographical errors.—G. W. MARTIN.

NOTES FOR STUDENTS

Metaxenia.—An interesting and clearly written article by NIXON[2] tells of his studies upon the immediate influence of pollen upon fruit size and time of ripening in the date palm. Experiments were carried on at Indio, California, using trees of the Deglet Noor variety as seed parents, and employing pollen from other varieties of *Phoenix dactylifera* and also pollen from *P. canariensis*. An ingenious technique was developed which permitted the use of various pollens on different strands of the same ovulate inflorescence. Fruits which had been pollinated with "Fard no. 4" colored about two weeks earlier than those with the pollen parent "Mosque." A striking display was produced where the two were side by side on the same bunch. By varying the pollen employed the experimenter was able to modify the size of fruit and time of ripening, but not the flavor or sugar content. In explanation of this immediate influence of pollen NIXON refers to papers by SWINGLE, read at the International Congress of Plant Sciences in 1926, and to a paper[3] by the same author just published. SWINGLE suggests that a hormone-like substance may be secreted by the development of endosperm or embryo, which, on reaching the ovary wall, influences further growth and development. SWINGLE thinks that metaxenia is not limited to the date palm but probably occurs in many plants, and that it may be exerted upon tissues outside of the seed and fruit. As knowledge of metaxenia develops it may be of great value to the horticulturist.—F. RAMALEY.

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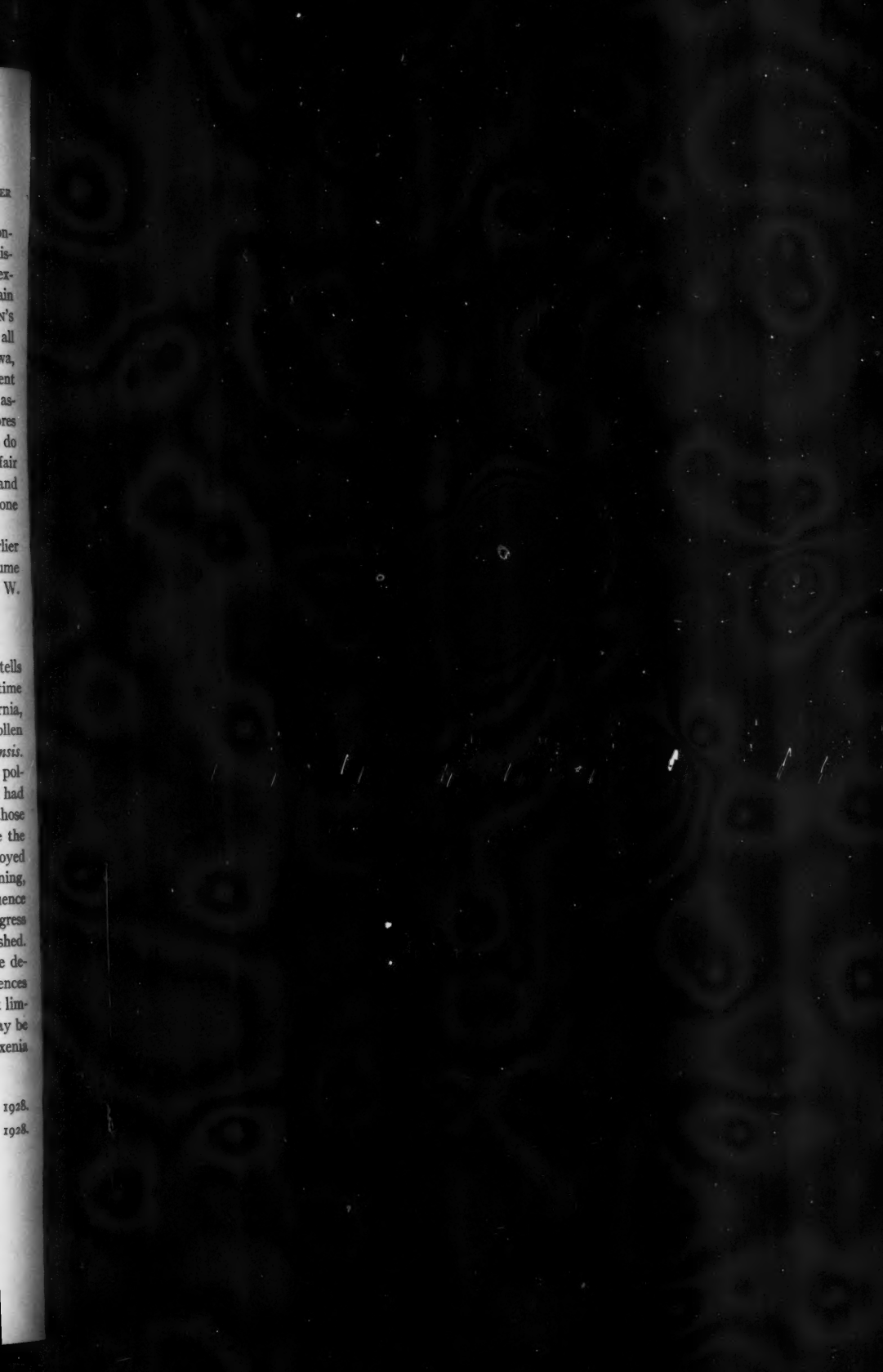
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